THE EVOLUTIONARY HISTORY OF REPRODUCTIVE STRATEGIES IN SCULPINS OF THE SUBFAMILY OLIGOCOTTINAE

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A

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Abstract

The sculpin subfamily Oligocottinae is a group of 17 nearshore species and is noteworthy for the fact that it contains both intertidal and subtidal species, copulating and non-copulating species, and many species with very broad geographic ranges. These factors, as well as the consistency with which the constituent genera have been grouped together historically, make the Oligocottinae an ideal group for the study of the evolution of a reproductive mode known as internal gamete association (IGA), which is unique to sculpins. I conducted a phylogenetic study of the oligocottine sculpins based on an extensive molecular dataset consisting of DNA sequences from eight genomic regions. From the variability present in those sequences, I inferred phylogenetic relationships using parsimony, maximum likelihood, and Bayesian inference. Results of these phylogenetic analyses show that some historical taxonomy and classifications require revision to align taxonomy with evolutionary relatedness. Specifically, the monotypic genus *Leiocottus* should be synonymized with *Clinocottus*; membership in the tribe Oligocottini should be reduced to include only the genera *Oligocottus*, *Clinocottus*, and *Orthonopias*; and the genus *Sigmistes* should be removed from the subfamily Oligocottinae. Using this new phylogenetic framework, I conducted an analysis of the evolution of reproductive behaviors and associated morphological characters in members of Oligocottinae. These traits were obtained through a critical review of the relevant literature and mapped on the phylogeny. Ancestral state reconstruction was used to explore their evolution. The results show that copulation and the presence of an enlarged male genital papilla are likely the ancestral states of Oligocottinae and that these characters were secondarily lost in the lineage composed of *Artedius corallinus*, *A. fenestralis*, *A. lateralis*, and *A. notospilotus*. The results also show that parental care in the group is split between the *Artedius* lineage, where males guard egg clutches, and the rest of the group, where egg guarding behavior is not present. I speculate that the differing ecology of these two groups has affected the evolution of reproduction and parental care in the subfamily, where subtidal lineages (*i.e.*, *Artedius*) engage in parental

care but have transitioned away from copulation, while the intertidal lineages maintained copulation but hide their eggs rather than guard them.

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Chapter 1: General introduction

1.1 Introduction

In this thesis I present a new phylogenetic study of oligocottine sculpins and use its results to infer the evolutionary history of reproductive traits in the group. The first chapter recapitulates the historical classification and taxonomy of oligocottine sculpins. It serves as a general introduction to the group and provides a broader context for the research and conclusions presented in Chapters 2 and 3. Chapter 2 describes a phylogenetic study designed as a stringent test of evolutionary relationships among oligocottine species using evidence from an extensive DNA sequence dataset. In Chapter 3, I use the inferred phylogeny and a likelihood framework to infer the evolution of these traits within the group. The results of this study show that the historical classification and taxonomy of some members of Oligocottinae are in need of revision. The results also strongly suggest that one group of oligocottine sculpins has secondarily lost the ability to copulate along with the physical traits often associated with copulation (i.e., an intromittent organ). These results are counter to some of the hypotheses of previous studies of the evolution of reproductive modes in these fishes, and highlight the importance of using a well-supported phylogenetic framework when investigating the evolutionary history of traits within a group.

1.2 Review of the systematics of Oligocottinae

The remainder of this chapter is a review of the historical classification and taxonomy of the members of the subfamily Oligocottinae. First, the classification history of Oligocottinae is reviewed. This includes both the formal delineation of Oligocottinae as well as the various evolutionary hypotheses posited by various authors. The strengths and weaknesses of our present understanding of oligocottine interrelationships are discussed. Second, the intra-generic relationships are reviewed for each constituent genus of Oligocottinae, as well as the genera that have been allied to the group. This review shows that members of Oligocottinae have been hypothesized to be closely related to one

another by numerous, independent studies. However, many of these studies relied on informal methods or on small datasets. I conclude that a stringent test of the monophyly and phylogenetic structure of Oligocottinae using a large dataset and modern methods of phylogenetic inference is needed.

The cottoids (superfamily Cottoidea) are a group of approximately 380 perciform fishes whose familial and sub-familial affinities have been the subject of much debate (e.g., Bolin 1944, Bolin 1947, Begle 1989, Imamura et al. 2005, Knope 2013), yet continue to be recognized as a largely natural grouping (e.g., Taranets 1941, Yabe 1985, Jackson 2003, Smith and Wheeler 2004). A parallel to this broader pattern can be found in the systematic of the subfamily Oligocottinae. Before Bolin (1944), the generic designations of oligocottine species were extremely unstable (see Bolin 1947 for discussion). Bolin's contributions produced a much more stable taxonomy for the group, however its alignment with phylogenetic relationships has been called into question by recent molecular-based studies (i.e., Ramon and Knope 2008, Knope 2013). Nevertheless, it is important to note that the vast majority of studies conclude that the oligocottine sculpins represent a natural or monophyletic group.

Prior to the designation of Oligocottinae as a subfamily, the "*Oligocottus* type" cottoids were thought to be closely related due to the following shared characters: the separation of the gill membranes from the isthmus, the presence of palatine teeth, and a body that was entirely scale-less, or possessed only rudimentary prickles (Greeley 1899). The "*Oligocottus* type" included all members of the modern genera *Oligocottus* Girard 1856 and *Clinocottus* Gill 1861, specifically: *O. rimensis* (Greeley 1899), *O. snyderi* Greeley 1898 (in Jordan and Evermann 1898b), *O. maculosus* Girard 1856, *O. rubellio* (Greeley 1899), *C. analis* (Girard 1858a), *C. recalvus* (Greeley 1899), *C. globiceps* (Girard 1858b), *C. embryum* (Jordan and Starks 1895), and *C. acuticeps* (Gilbert 1896).

The subfamily Oligocottinae was formally described in 1926 based on the characters shared by "*Oligocottus* type" sculpins (*sensu* Greeley 1899) plus three additional characters: three soft pelvic rays, a "moderate number" of dorsal spines, and preopercular spines without antler-like processes, as well as the stipulation that the

palatine teeth were in "bands" (Hubbs 1926b). The species contained in Oligocottinae were all of the "*Oligocottus* type" species with the addition of *Sigmistes caulias* Rutter 1898 (in Jordan and Evermann 1898b). The species were partitioned into two tribes: the Oligocottini, consisting of the species that today make up the genus *Oligocottus*, and the Clinocottini, containing all of the species in the modern genus *Clinocottus* plus *S. caulias*. Hubbs noted that there existed a close relationship between the Oligocottinae and the *Pseudoblennius*-type cottoids.

Taranets (1941) re-described Oligocottinae as "the genera related to Pseudoblenniinae but [differing] from the latter by having:" upper pharyngeal teeth on two plates on each side, the pelvic fin formula (I,3), and by "other characteristics" which were not described. Interestingly, the published literature is uninformative on the relationship between Hubbs' contributions to oligocottine systematics and those of Taranets. Taranets (1941) does not reference Hubbs' work in his description of the group. The subfamily was divided into two "generic groups" which will hereafter be referred to as tribes: the Oligocottini, which contained all of the members of Oligocottini and Clinocottini (*sensu* Hubbs 1926b) lumped together, along with the tribe Artediini, which contained the species *Orthonopias triacis* Starks and Mann 1911 and all the species that make up the modern genus *Artedius* Girard 1856, specifically: *A. harringtoni* (Starks 1896), *A. corallinus* (Hubbs 1926a), *A. fenestralis* Jordan and Gilbert 1883, *A. notospilotus* Girard 1856, and *A. lateralis* (Girard 1854a).

The monotypic genus *Leiocottus* Girard 1856 was inferred to be closely related to *Clinocottus* (Bolin 1944, 1947; Fig. 1.1). This species "complex" (*i.e. Artedius* + *Orthonopias* + *Oligocottus* + *Clinocottus* + *Leiocottus*, hereafter referred to as the A-Or+O-C-L clade) was united by a tendency toward reduction of both the preopercular spines and squamation, with the distinct *Artedius*-*Orthonopias* line distinguished by its *Hemilepidotus*-like dorsal scale band, and the *Oligocottus*-*Clinocottus*-*Leiocottus* line distinguished by its fragmentation or complete loss of scales. Bolin (1944, 1947) did not designate the group as a subfamily due to a concern that subfamilies that had been delineated at that time were "based on insufficient grounds or upon a distribution of

characters that [did] not follow major evolutionary lines." It should be noted that the analysis was restricted to taxa that occurred in California and, as such, the placement of the genus *Sigmistes* was not considered.

The Aleutian archipelago endemic species *Phallocottus obtusus* (Schultz 1938) was allied to the genus *Sigmistes* Rutter 1898 (in Jordan and Evermann 1898b), which was allied to *Clinocottus* (part of the A-Or+O-C-L clade) based on meristic data (Howe and Richardson 1978). However, the authors of this study provided little to no evidence as justification for their assessment of affinities (outside of tables detailing the range and frequency of their meristic data), and in the introduction to the manuscript, the authors explicitly stated that the assessments were "presented as aids but should be considered preliminary, tentative and unpublished."

Patterns of variation in larval characteristics of preopercular spines, ontogenetic development, and osteology have provided further support for the *Artedius+Clinocottus-Oligocottus* grouping (Richardson 1981, Washington 1986). However, these larval traits have yet to be described in *Orthonopias*, *Leiocottus*, *Sigmistes*, or *Phallocottus*, and thus offer no insight onto the phylogenetic placement of those genera. An analysis of larval stages in *Artedius*, *Clinocottus*, *Oligocottus* and a variety of outgroup taxa identified the following three larval synapomorphies to unite the A+C-O clade (Fig. 1.2): multiple (>4) preopercular spines, enlargement and expansion of the neural arches, and first three neural arches unfused (Washington 1986). However, as in all previously discussed studies, the validity of this grouping was not tested with objective methodologies. Instead, the groupings were proposed based on the observations and *ad hoc* weighting of characters based on no explicitly stated rationale. Therefore, it is difficult or impossible to reproduce those results or assess the strength of their conclusions. Modern approaches to inferring phylogenetic relationships rely on formalized, reproducible analytical protocols that allow testing of hypotheses of phylogeny.

The first such modern study of any oligocottine sculpin was a cladistic study focused on the interspecific relationships within the genus *Artedius* (Begle 1989). Fifty-three morphological characters were compared among *A. fenestralis*, *A. notospilotus*, *A.*

lateralis, *A. corallinus*, *A. harringtoni*, *A. creaseri* (Hubbs 1926b), and *A. meanyi* Jordan and Starks 1895. That analysis also included the genera *Orthonopias, Oligocottus, Clinocottus, Chitonotus* Lockington 1879*, Hemilepidotus* Cuvier 1829*,* and *Icelinus* Jordan 1885 as outgroup taxa used to determine the ancestral and derived states of the characters. The results showed that *Artedius* was in fact a polyphyletic genus that included two distinct clades (Fig. 1.3). As such, the genus *Ruscarius* Jordan and Starks 1895 was resurrected with the two species it once contained: *Ruscarius meanyi* and *R. creaseri* (formerly *A. meanyi* and *A. creaseri*). Additionally, the results provided only a single synapomorphy (ossification of the opercle) uniting *Oligocottus, Clinocottus*, and the redefined *Artedius* clade (Fig. 1.3).

Strauss (1993) combined the larval character dataset of Washington (1986) with the *Artedius*-centric dataset of Begle (1989) and performed a parsimony analysis on the combined data. This full dataset included members of *Oligocottus*, *Clinocottus*, *Artedius*, *Ruscarius*, and two outgroup taxa (Fig. 1.4). His results showed strong support for the monophyly of Oligocottinae, with six synapomorphies uniting the group (larval: greater than 5 preopercular spines, the pattern of preopercular spines, modified parietal spines, trailing hindgut; adult: pelvic fins supported by one spine and three rays, and incomplete ossification of the opercle). However, the author noted that Washington (1986) found only two synapomorphies for this group (larval: greater than five preopercular spines, trailing hindgut), and Begle (1989) found only one (see above). Thus, the additional synapomorphies in the combined dataset were likely an artifact of the loss of many of the outgroup taxa used in the previous studies, which could not be included in the combined analysis due to limits in the taxonomic overlap between the two studies. The author also noted that the evidence for a sister relationship of *Oligocottus* and *Clinocottus* was weak and came solely from characters found in Begle (1989), where the two genera were included as outgroup taxa. Thus, Strauss (1993) concluded that the relationship between *Artedius*, *Clinocottus*, and *Oligocottus* could not be resolved by his study.

Large-scale morphological studies (*i.e.*, Yabe 1985, Fig. 1.5; Jackson 2003, Fig. 1.6) have failed to resolve the relationships among the genera of Oligocottinae. However,

two recent DNA sequence based studies (Ramon and Knope 2008 and Knope 2013, Figures 1.7 and 1.8, respectively) have supported Bolin's hypothesis of a close relationship between *Oligocottus, Orthonopias, Artedius, Clinocottus, Leiocottus,* and *Ruscarius*. Like Bolin (1944), they did not include any members of the genera *Sigmistes* or *Phallocottus*. Unlike Bolin (1944), results of these studies agree with the distinction of *R. meanyi* and *R. creaseri* separate from *Artedius*, as suggested by Begle (1989). Unlike any other previous phylogenetic study however, they called into question the monophyly of *Oligocottus* and *Clinocottus*.

Ramon and Knope (2008) compared two mitochondrial loci (NADH1 and Cyt *b*) and one nuclear locus (S7 intron 1) across all members of the genera *Oligocottus*, *Clinocottus*, *Artedius*, *Ruscarius* (*sensu* Begle 1989), *Orthonopias* (monotypic), and *Leiocottus* (monotypic), as well as a variety of outgroup taxa (e.g., *Enophrys bison* (Girard 1854b), *Chitonotus pugetensis* (Steindachner 1876), *Leptocottus armatus* Girard 1854b, *Stellerina xyosterna* (Jordan and Gilbert 1880), and *Rhamphocottus richardsonii* Günther 1874). The sequences from the three genomic regions were concatenated and a phylogenetic inference was conducted from the combined dataset using maximum likelihood, Bayesian inference, and parsimony. Their results supported the monophyly of *Ruscarius* and *Artedius* (in agreement with Begle 1989), but suggested that *Clinocottus,* as currently defined, is polyphyletic because *C. analis* forms a clade with *L. hirundo* and *C. acuticeps* is the sister lineage of the *Artedius* clade (Fig. 1.7). The monophyly of *Oligocottus* (*sensu* Bolin 1944) was also called into question as *Orthonopias triacis* was nested within *Oligocottus*. However, support values for the clade consisting of *Oligocottus* + *Orthonopias* were very low. In these aspects, the results of Ramon and Knope (2008) conflict with the classification proposed by Bolin (1944), but otherwise support the monophyly of the A-Or+O-C-L clade.

Knope (2013) compared DNA sequences from a mitochondrial locus (Cyt b) and a nuclear locus (S7 intron 1) across 99 species of North Pacific cottoids. The resulting phylogenetic hypothesis regarding the A-Or+O-C-L taxa (Fig. 1.8) broadly agrees with that of Ramon and Knope (2008), with the following notable exceptions: *C. acuticeps* is

nested within the *Artedius* clade rather than as a sister group to it, and *Orthonopias triacis* is placed as the basal-most member of a clade that also contains *C. embryum*, *C. globiceps*, and *C. recalvus,* rather than nested within *Oligocottus*.

1.3 Current issues regarding classification and taxonomy of Oligocottinae

The primary source for the modern taxonomy of the oligocottine sculpins is the hypothesis advanced by Bolin (1944, 1947). Since then, the only cladistic analyses of relationships within the group were those of Begle (1989) and Strauss (1993). The former was restricted to species of the genus *Artedius* and treated allied genera as outgroups. Begle (1989) remains the last published taxonomic revision of any member of Oligocottinae. However, several methodological errors in that study cast doubt onto the accuracy of the results. For instance, variable morphological character states within the outgroup taxa are coded inconsistently. For example, within the genus *Oligocottus* there are both species that have external scales and species that do not. In the data matrix of Begle (1989), *Oligocottus* is coded as having a scale-less body in character #20, yet it is coded as having head scales that are indistinguishable from its body scales in character #13. The follow-up study by Strauss (1993) did not modify any of the Begle (1989) character states, and extrapolated those generic-level states to all species within the genus For example, *Clinocottus* is coded as having a scale-less body in Begle (1989) and *Clinocottus analis* is coded as having a scale-less body in Strauss (1993), when in fact *C. analis* has external scales (see description in Bolin, 1944). These flaws call into question the conclusions of these studies and lend justification to a reevaluation of oligocottine systematics.

The DNA sequence based studies Ramon and Knope (2008) and Knope (2013) included broad taxon samples but low samples sizes (n = 1-3) for many wide-ranging species (*e.g.*, *Oligocottus maculosus* is found along the NE Pacific coast from central California to the Alaskan Peninsula, yet is represented by only two individuals collected from a single location in Oregon), and few (3 and 2, respectively) loci. In both studies, the sequence data were analyzed as a concatenated alignment with no allowance for differing evolutionary dynamics between loci. The use of an oversimplified model of

molecular evolution can increase the incidence of artifacts like long-branch attraction and ultimately result in an inaccurate phylogenetic hypothesis (see Sullivan and Swofford 1997). When combining mitochondrial protein-coding genes and nuclear introns into a single dataset for the purposes of phylogenetic inference, a partitioned analysis has been shown to be more appropriate than an unpartitioned analysis (McGuire et al. 2007). In fact, both Ramon and Knope (2008) and Knope (2013) noted that the optimal model for the nuclear S7 locus was different from the optimal model for the mitochondrial gene(s), yet neither of the manuscripts addressed or justified their lack of partitioning. This may account for some of the extremely unorthodox relationships proposed in Knope (2013) (*e.g.*, sister-grouping of *Scorpaenichthys marmoratus* and *Rhamphocottus richardsonii*; extreme paraphyly of the genus *Nautichthys*; the placement of *Leptocottus armatus* as sister lineage of the entire cottoid radiation). Knope (2013) examined all of the cytochrome b and S7 sequences reported in Ramon and Knope (2008), and both studies relied on Maximum Likelihood and Bayesian inferences, yet their resulting trees differ in the resolution of the genera *Artedius* and *Oligocottus*, and the placement of *Orthonopias triacis*, *C. acuticeps,* and the *C. analis* + *L. hirundo* clade (see Figures 1.7 and 1.8). A further point of concern in Knope (2013) is the apparent lack of familiarity with the morphology and descriptions by Bolin (1944) of some of the species (e.g., incorrectly quoting Bolin's characterization of *C. embryum* as having a "large rounded head," thus supporting Knope's monophyletic grouping of *C. embryum*, *C. globiceps*, and *C. recalvus*, while, according to Bolin (1944), the latter two have a "very bluntly rounded, hemispherical" head while *C. embryum* has a "moderately pointed and angular, definitely not hemispherical" head). Altogether, weaknesses evident in the studies reported to date justify continued efforts to improve our understanding of the phylogeny of Oligocottinae.

1.4 Taxonomic history of the constituent genera of Oligocottinae

For the purposes of this study, I assume that any currently recognized genus that at any point has been placed within Oligocottinae or allied to a genus that has been placed within Oligocottinae is a candidate for inclusion within the subfamily. Those genera are:

Artedius, Clinocottus, Leiocottus, Oligocottus, Orthonopias, Phallocottus, Ruscarius, and *Sigmistes*. Below, I review the taxonomic history of each of these genera.

1.4.1 *Artedius* Girard 1856

The taxonomic history of *Artedius* was thoroughly and most recently reviewed by Begle (1989). However, it is important to note that species groupings suggested in the earlier work of Richardson (1981) were based on subjective assessments of morphological similarity, did not involve explicit character state coding or a formal clustering analysis. Similarly, Washington (1986) searched for characters to delineate groups among and within the genera *Oligocottus*, *Clinocottus*, and *Artedius* (A-C-O), but did not employ cladistic methods. In fact, a strict consensus tree based on parsimony analysis of the traits reported by Washington (1986) is only congruent with a monophyletic A-C-O clade (Strauss 1993) and is incompatible with all other relationships advanced by Washington (1986).

Begle (1989) conducted a cladistic analysis of 53 morphological characters among the members of *Artedius* as well as six outgroup taxa. His results provided no support for the inclusion of *A. meanyi* and *A. creaseri* within the *Artedius* clade, but did support a close relationship between the two species. To reflect this result the genus *Ruscarius* was resurrected for *R. meanyi* and *R. creaseri*. Begle's study concluded that the remaining species made up a redefined *Artedius* that could be diagnosed by the following six synapomorphies: postcleithra absent; two or three parallel rows of scales closely confined to base of axilla; no cirri above axilla; triangular flange at posterior end of pterotic extending to margin of cranium; ventral surface of chin covered with circular areas of lighter pigmentation interrupting the generally darker background; and darker background pigmentation of lateral body surface interrupted by circular areas without pigment. Begle also synonymized *A. hankinsoni* (Hubbs 1926a), known from only the type specimen, after considering it an aberrant form of *A. lateralis*. Results of DNA sequence-based studies (Ramon and Knope 2008, Knope 2013) support this composition of *Artedius*.

1.4.2 *Clinocottus* Gill 1861

The genus *Clinocottus* was first established by Gill (1861) when he broke apart *Oligocottus* (*sensu* Girard 1856) and erected a distinct genus for each of its three constituent species. The type species, *O. maculosus*, remained in the newly monotypic *Oligocottus*, while *O. analis* was placed in the newly created *Clinocottus* based on "the absence of palatine teeth, the presence of prickles on the body, the entire anal fin and the form of the head." Finally, *O. globiceps* was designated as *Blennicottus globiceps* based on "the form of the head, its armature, and the structure of the anal fin." Jordan and Starks (1895) described a new species of *Oligocottus*, *O. embryum*, and made a distinction between northern and southern forms of *B. globiceps*. The northern form was distinguished by an abundance of head cirri and became *B. globiceps bryosus*, while the southern form, distinguished by considerably less head cirri was designated *B. globiceps globiceps.*

Clinocottus acuticeps was described as *Oligocottus acuticeps* by Gilbert (1896). Jordan and Evermann (1898a) elevated *O. acuticeps* to generic distinction as the type species for a new genus, *Oxycottus*, which they allied to *Oligocottus* but noted that it differed in lacking an "upward process on the sharp, upwardly curved preopercular spine" and also lacked a "slit behind [the] last gill." *Oxycottus embryum* (formerly *Oligocottus embryum*) was added as a congener. Jordan and Evermann (1898b) reexamined the designation of *Oxycottus* as a separate genus and opted to designate it a subgenus of *Blennicottus* based on the shared trait of a lack of slit behind the last gill. The revised *Blennicottus* contained *B. globiceps, B. acuticeps*, and *B. embryum*.

Greeley (1899) abolished the subspecies *B. globiceps bryosis* as it had become applied to the form originally described as *B. globiceps* and reapplied the description of *B. globiceps* to the northern form (from which it had been originally described), and named the southern form *B. recalvus*. Greeley (1899) makes no mention of the taxonomic revision of *Oxycottus* to a sub-genus of *Blennicottus* by Jordan and Evermann (1898b), and retains it at the generic rank, with *O. acuticeps* and *O. embryum* as its constituent species.

Hubbs (1926b) split *Oxycottus* and removed *O. embryum* to a new genus as *Allocottus embryum*. He also renamed *B. recalvus* as the monotypic *Montereya recalva*, split *Clinocottus analis* into *C. analis analis* and *C. analis australis* (northern and southern forms, respectively), and synonymized *Rusulus saburrae* Starks and Mann (1911), which was known from only a single specimen, with the northern form.

Bolin (1944) disregarded all subspecific designation within the *Clinocottus analis* complex and moved *Allocottus embryum, Montereya recalva, Blennicottus globiceps*, and *Oxycottus acuticeps* to *Clinocottus* with *C. analis*.

Clinocottus (*sensu* Bolin 1944) remains a natural group in results of the larval studies conducted by Richardson (1981) and Washington (1986), as well as the cladistic analysis of Strauss (1993) based on a concatenation of the datasets of Washington (1986) and Begle (1989). The DNA-based studies of Ramon and Knope (2008) and Knope (2013) have called into question the monophyly of *Clinocottus* but no proposed changes to taxonomy were made from those results.

1.4.3 *Leiocottus* Girard 1856

Leiocottus hirundo was described by Girard (1856). The genus has remained monotypic and unchanged since its original description.

1.4.4 *Oligocottus* Girard 1856

Oligocottus was described by Girard (1856) with the description of *O. maculosus*. Girard (1858b), Jordan and Starks (1895) and Gilbert (1896) all described new species of *Oligocottus* (*O. analis*; *O. globiceps* and *O. embryum*; and *O. acuticeps*, respectively), but each of these would ultimately be moved to *Clinocottus* as described above.

Jordan and Snyder in Jordan (1896) described *Oligocottus borealis*, which they noted was closely related to *O. maculosus*. Greeley in Jordan and Evermann (1898b) named a new species of *Oligocottus*, *O. snyderi*, but offered no description other than, "new species," and a reference to "Greeley, M.S. 1898." Greeley (1899) offered a description of the new species, but under a new genus, as *Dialarchus snyderi*. Oddly, *Dialarchus* differed from *Oligocottus* "only in the character of anal rays of male," yet

was given generic distinction. Greeley (1899) also described *Eximia rubellio* and *Rusciculus rimensis*. Hubbs (1926b) rechristened *E. rubellio* as *Greeleya rubellio* because the genus name *Eximia* was already in use. Taranets (1941) lists *Eximia* and *Greeleya* under the synonyms of *Dialarchus*, but offers no justification for this synonymy. No other published work presents that arrangement.

Bolin (1944) united *Greeleya rubellio*, *Dialarchus snyderi*, and *Rusciculus rimensis*, with *Oligocottus maculosus* under the genus *Oligocottus*. Ramon and Knope (2008) called into question the monophyly of *Oligocottus* (*sensu* Bolin 1944) but did not propose nomenclatural changes.

1.4.5 *Orthonopias* Starks and Mann 1911

Orthonopias triacis was described by Starks and Mann (1911). The genus has remained monotypic and unchanged since its original description.

1.4.6 *Phallocottus* Schultz 1938

Phallocottus obtusus was described by Shultz (1938). This genus has remained monotypic and unchanged since its original description.

1.4.7 *Ruscarius* Jordan and Starks 1895

Ruscarius meanyi was described by Jordan and Starks (1895). *Ruscariops creaseri* was described by Hubbs (1926a). These two species were the types of respective monotypic genera until Bolin (1944) synonymized *R. creaseri* into his redefined *Artedius*. Bolin (1944) did not include *R. meanyi* in either his analysis or reclassification. Rosenblatt and Wilkie (1963) re-described *R. meanyi* and reclassified it as *Artedius meanyi*. Begle (1989) conducted a cladistic analysis of *Artedius* (*sensu* Bolin 1944) and concluded that, while there was evidence uniting *A. meanyi* and *A. creaseri* together, there was no evidence uniting them with the rest of *Artedius*. Begle resurrected *Ruscarius* to contain *R. meanyi* and *R. creaseri*.

1.4.8 *Sigmistes* Rutter 1898

Sigmistes caulias was described by Rutter in Jordan and Evermann (1898b). The only other member of this genus, *S. smithi,* was described by Schultz (1938). The genus has remained otherwise unchanged since its original description.

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I would like to thank my advisor, J. Andrés López, as well as Derek Sikes for their guidance in all things nomenclatural. I would also like to thank W. Leo Smith for aiding me in my search for many hard to find manuscripts and for sharing his great knowledge of cottoid evolution with me.

1.6 Figures

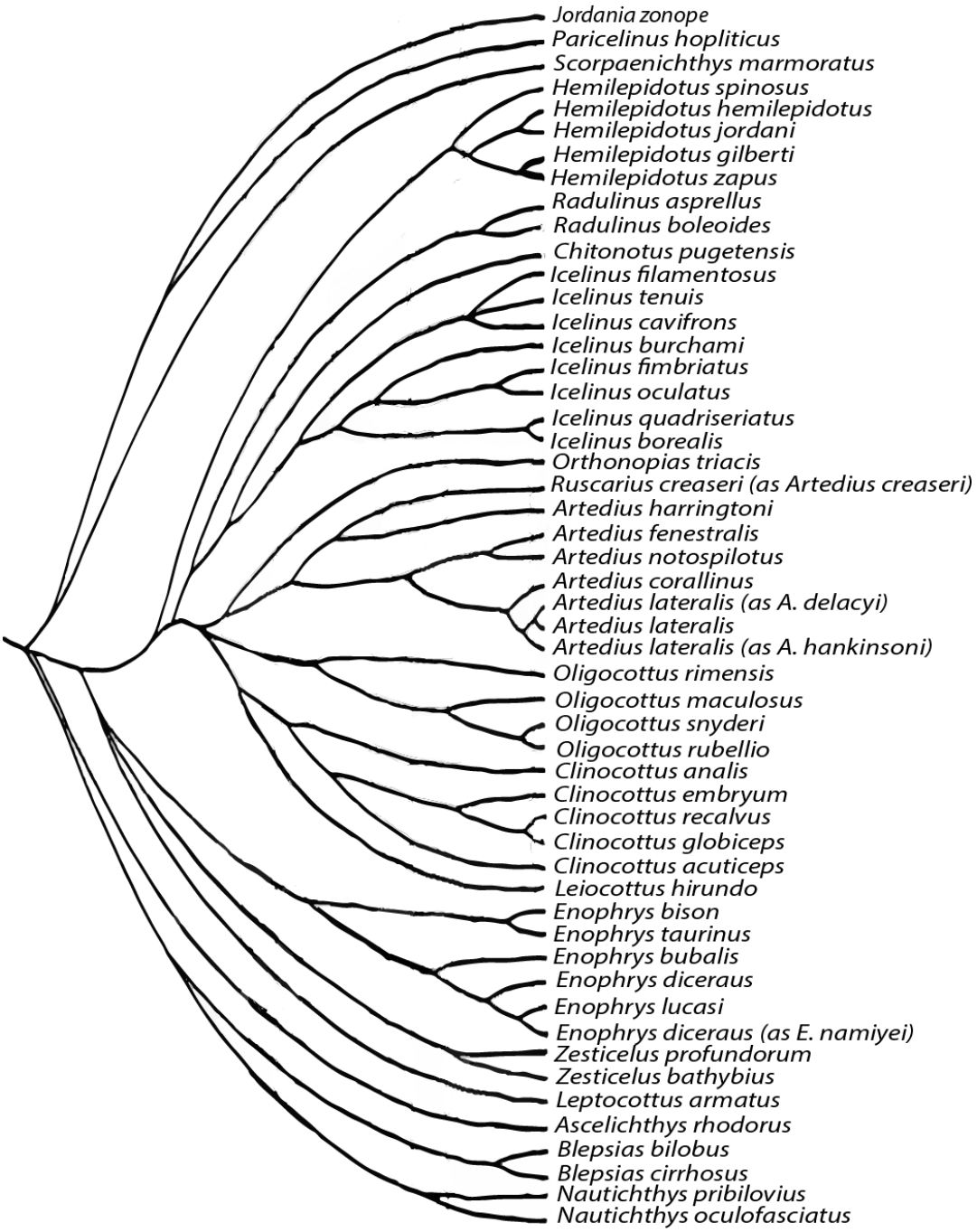

Figure 1.1: Bolin's (1947) phylogeny of marine cottoids of California. Hand drawn to show relationships proposed by Bolin (1944, 1947). Phylogeny was inferred using

physical examination of external and osteological characters. Adapted from Bolin (1947: Figure 1).

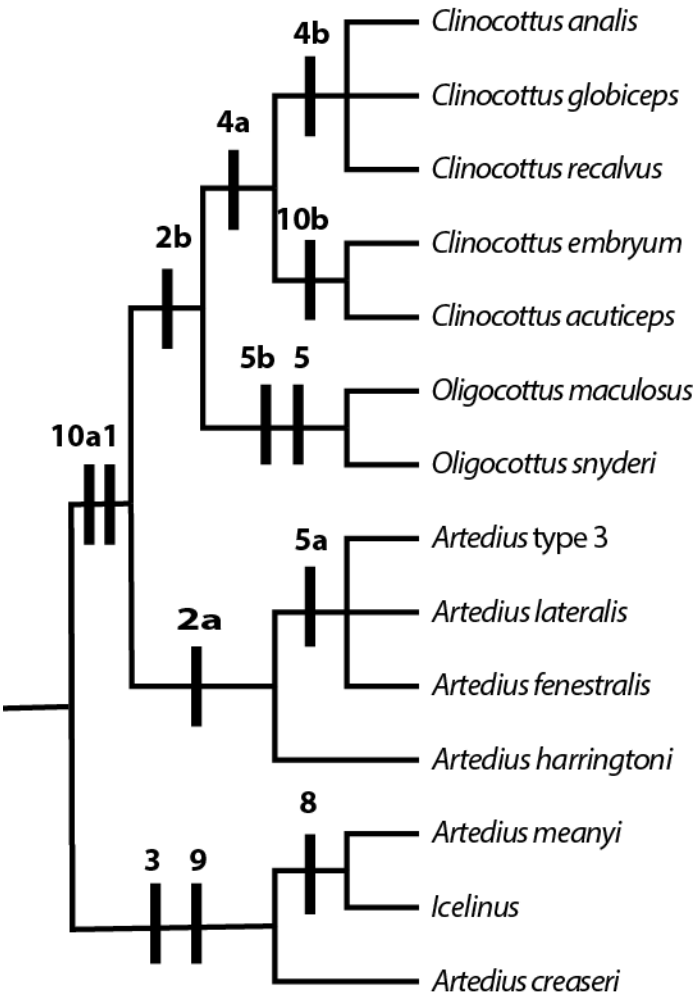

Figure 1.2: Washington's (1986) cladogram of 14 cottid taxa. Evolutionary relationships were inferred based on 10 larval morphological characters. Adapted from Washington (1986: Figure 5). Synapomorphies for each clade are indicated at the nodes. 1: multiple preopercular spines. 2: "*Artedius*" spine pattern. 3: dorsal spine longest. 4a: auxiliary spine (one). 4b: auxiliary spine (two). 5: nape bubble. 6a: dorsal gut diverticula. 6b: dorsal gut bumps. 8: 2 pelvic fin rays. 9: pointed snout. 10a: trailing gut. 10b greatly trailing gut. See Washington (1986) for more detail.

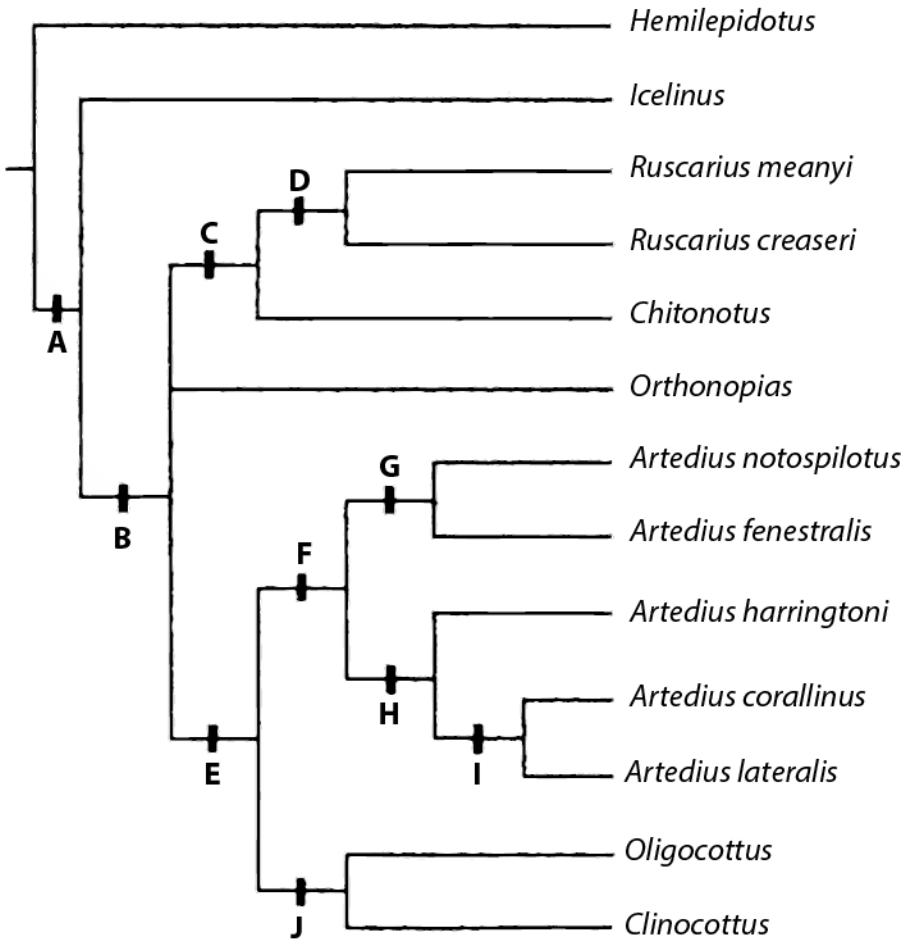

Figure 1.3: Begle's (1989) phylogeny of *Artedius*. Phylogeny was based on a consensus cladogram generated from parsimony analysis of 53 morphological characters from *Artedius* and several outgroup taxa. Adapted from Begle (1989: Figure 1). Synapomorphic characters are indicated at nodes. A: 7 (number of pelvic rays), 32 (scale ridge placement). B: 7 (number of pelvic rays), 53 (presence of anterior teeth on scale ridge). C: 5 (scales on snout), 42 (scales on eye), 44 (shape of scale ridge), 51 (scale ridge attachment). D: 3 (body color), 4 (scales above axilla), 6 (upper preopercular spine), 10 (male color), 26 (scales under orbit), 28 (preorbital cirri). E: 41 (ossification of opercle), 52 (width of scale ridge). F: 25 (loss of post-cleithra), 27 (scales behind axilla), 29 (cirri above axilla), 40 (pterotic flange), 46 (chin pigmentation), 49 (body pigmentation). G: 11 (mandibular pores), 12 (lateral line pores), 13 (head scales), 17 (nasal pores), 39 (cirri on nasal spine). H: 45 (distribution of scale ctenii), 48 (branchiostegal pigmentation). I: 15

(cirri on suborbital stay), 47 (extra spots on chin), 50 (extra spots on body). J: 20 (squamation). See Begle (1989) for specific character states and discussion.

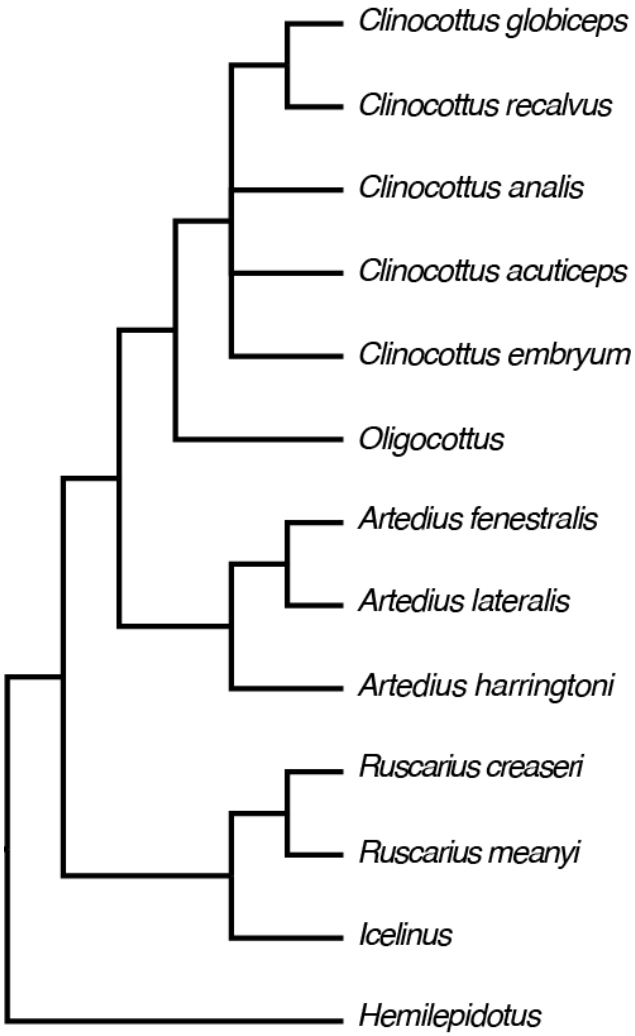

Figure 1.4: Strauss' (1993) phylogeny of *Artedius*, *Clinocottus*, and *Oligocottus*. Phylogeny was based on a strict consensus cladogram generated from a parsimony analysis of larval (n=10) and adult (n=37) characters, taken from Washington (1986) and Begle (1989), respectively. Taxa included were only those shared by both studies. Adapted from Strauss (1993: Figure 3).

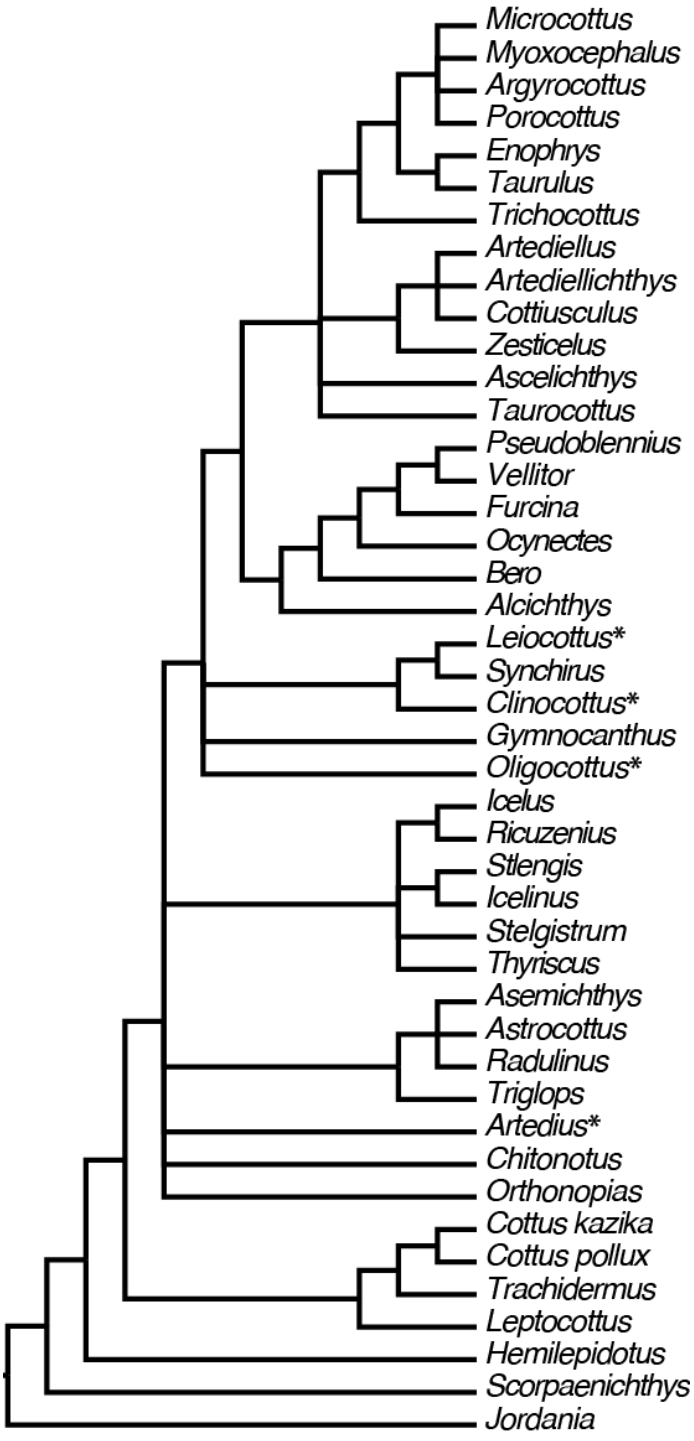

Figure 1.5: Yabe's (1985) phylogeny of 44 cottid taxa. Adapted from Yabe (1985: Figure 58). Relationships determined through analysis of 60 morphological characters.

Cladistic methodology was used for analysis, but methods for determining the most optimal tree were not explicitly stated. See Yabe (1985) for greater detail. For clarity, oligocottine taxa are indicated by asterisk.

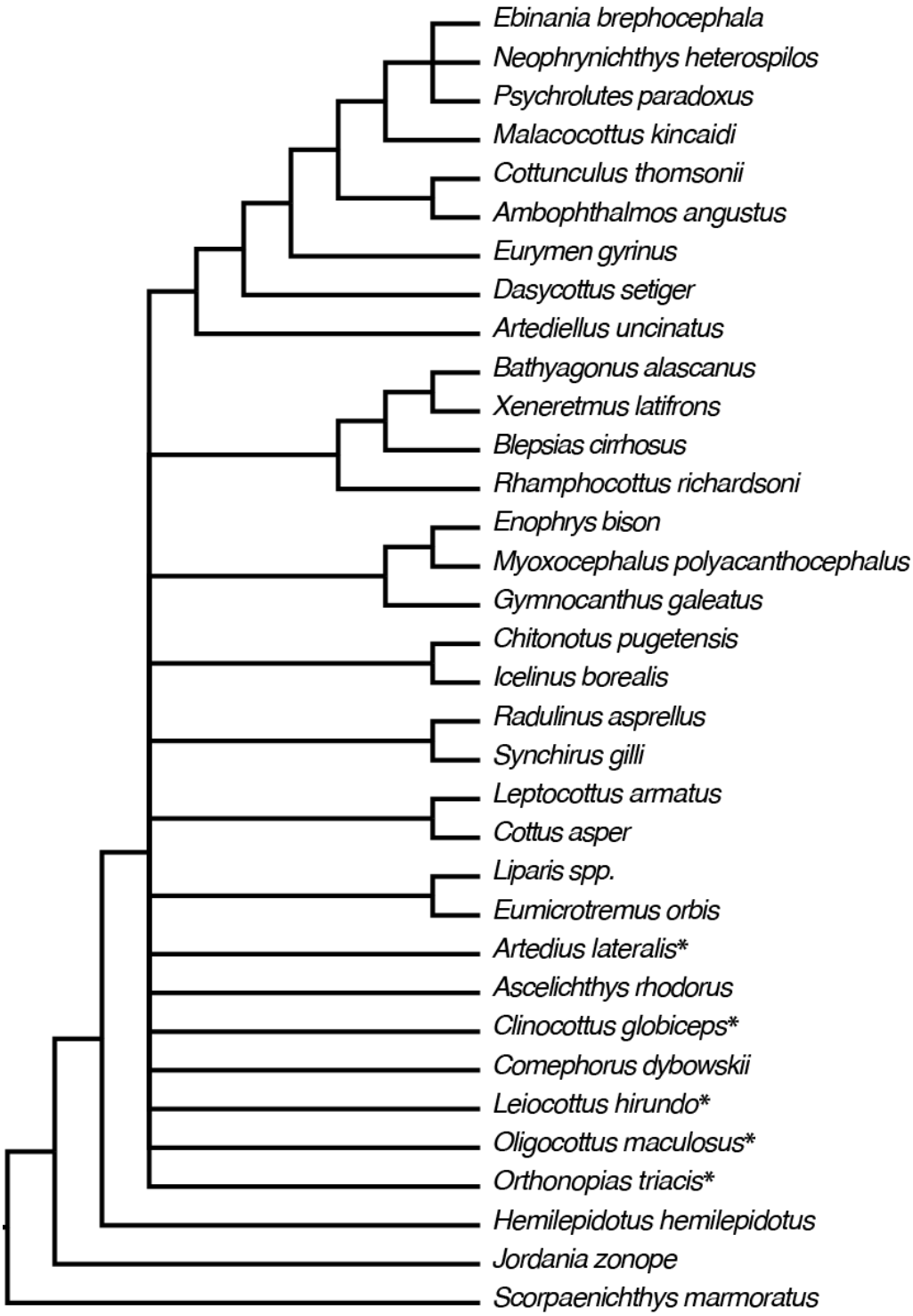

Figure 1.6: Jackson's (2003) phylogeny of Cottoidei. Adapted from Jackson (2003: Figure 3-16). Phylogeny determined by strict consensus of 96 equally most parsimonious

trees based on 68 morphological characters with 111 minimum steps of evolution. For clarity, oligocottine taxa are indicated by asterisk.

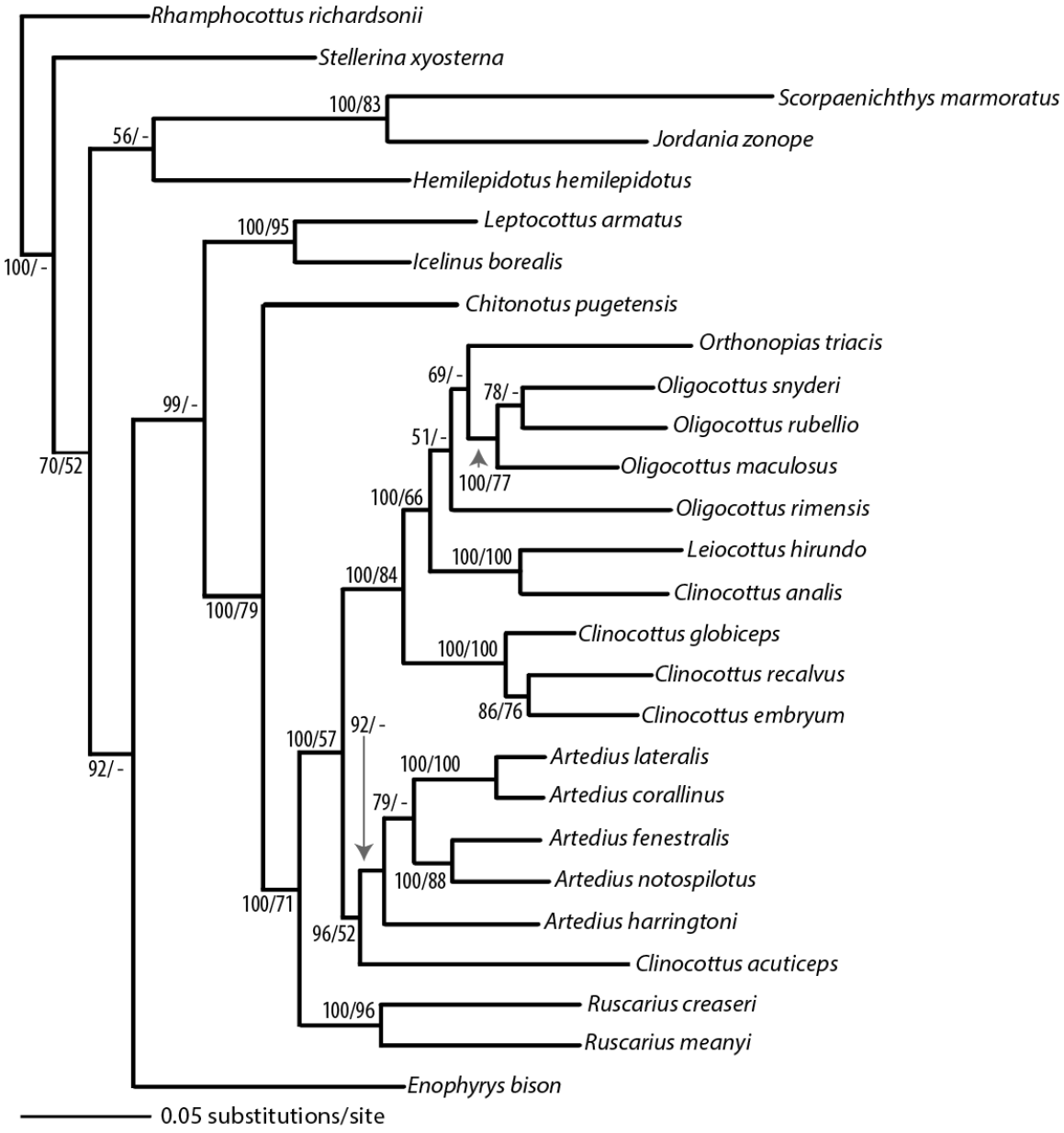

Figure 1.7: Ramon and Knope's (2008) phylogeny of several oligocottine taxa. Phylogenetic reconstruction based on Bayesian analysis of concatenated mitochondrial (cyt *b* and NADH) and one nuclear (S7 intron) DNA sequences. Adapted from Ramon and Knope (2008: Figure 1). Bayesian posterior probabilities and maximum likelihood bootstrap support are indicated at each node. Dash mark in place of a support value indicates less than 50% support by an analysis for a given node.

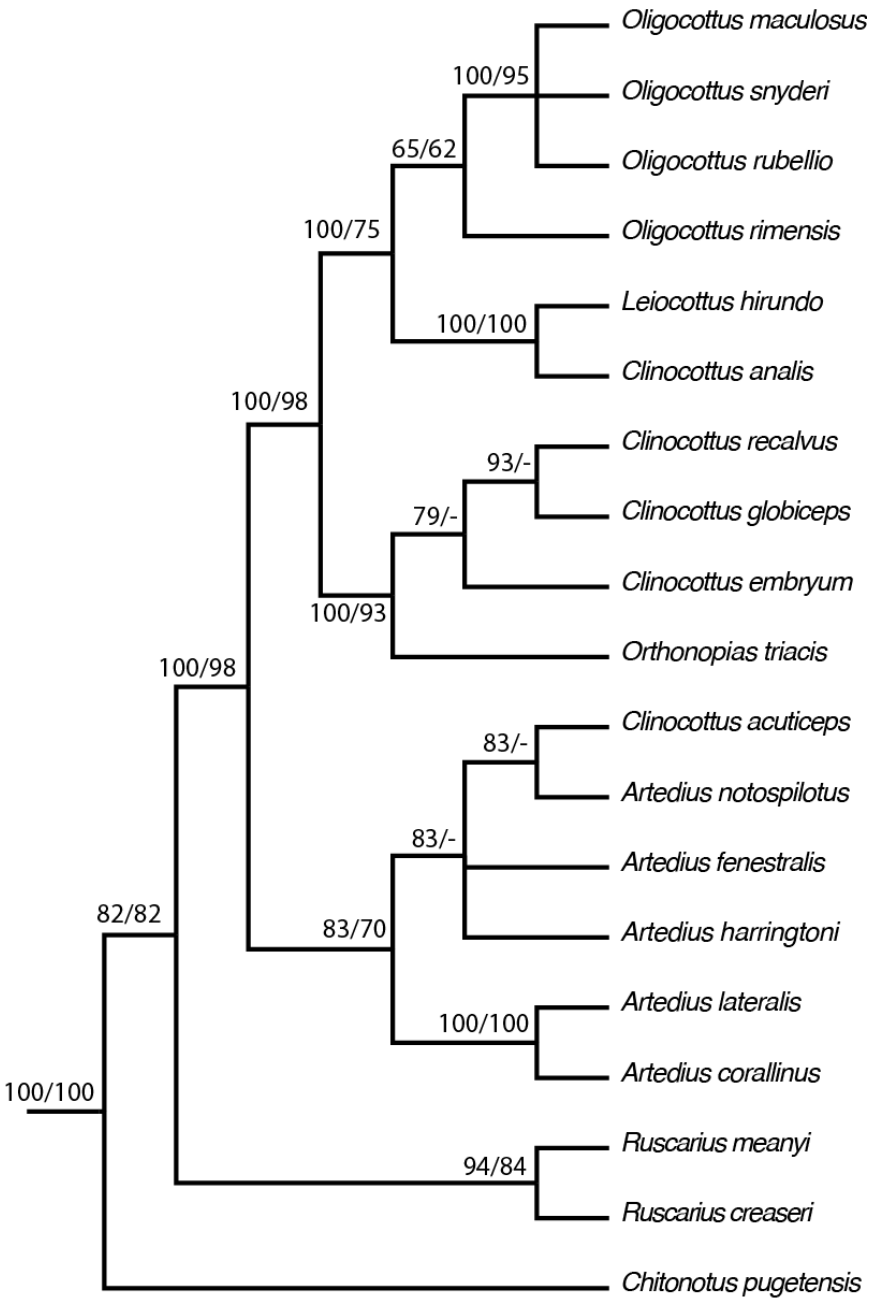

Figure 1.8: Knope's (2013) phylogeny of several oligocottine sculpins. Cladogram based on Bayesian phylogenetic reconstruction of North Pacific sculpins, adapted from Knope (2013: Figure 1). Phylogeny inferred by Bayesian and maximum likelihood analyses of a concatenated mitochondrial (cyt *b*) and nuclear (S7 intron) DNA sequences. Support values are indicated at each node (Bayesian posterior probabilities and bootstrap support

for maximum likelihood analysis, respectively), dash mark in place of a support value indicates less than 50% support by an analysis for a given node.

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Chapter 2: Molecular phylogenetics of sculpins of the subfamily Oligocottinae (Cottidae)[1]

2.1 Abstract

I conducted a phylogenetic study of 31 species of true sculpins (Cottidae, Perciformes), using evidence from the DNA sequences of eight genomic regions to test the makeup of the sculpin subfamily Oligocottinae. Additionally, 6 species representing the families Agonidae, Hemitripteridae, Hexagrammidae, and Rhamphocottidae were included as outgroup taxa. Sequence data were examined in maximum parsimony, maximum likelihood, and Bayesian phylogenetic inference frameworks. Results of these phylogenetic analyses show that a systematic revision of the group is warranted. In particular, *Leiocottus hirundo* should be included in the genus *Clinocottus* as *C. hirundo*; the composition of the tribe Oligocottini should be revised to include only the genera *Oligocottus*, *Clinocottus*, and *Orthonopias*; and the genus *Sigmistes* should be excluded from the subfamily Oligocottinae. A further finding is that, while the genera *Artedius* and *Oligocottus* were shown to be monophyletic, the genus *Clinocottus* is a polyphyletic assemblage including at least three distinct evolutionary lines with closer affinities to other oligocottine lineages than to one another: the subgenus *Blennicottus*, the subgenus *Clinocottus*, and the species *Clinocottus acuticeps*.

2.2 Introduction

The subfamily Oligocottinae comprises 18-20 species of nearshore sculpins (family Cottidae Bonaparte 1831) that range along the north Pacific coast from the Baja Peninsula in Mexico to the Kuril Islands in Russia (Hubbs 1926b, Taranets 1941, Masuda and Muzik 1992, Mecklenburg et al. 2002). This group includes many intertidal species and is remarkable for the diversity of coloration and reproductive specializations found among its constituent taxa. The subfamily was first delineated to include the members of the currently accepted genera *Oligocottus* Girard 1856, *Clinocottus* Gill 1861, and *Sigmistes* Rutter 1898 (in Jordan and Evermann 1898) (Hubbs 1926b). It was later

[1] Buser, T.J. and López, J.A. In prep. Molecular Phylogenetics and Evolution.

expanded with the inclusion of the species currently assigned to the genera *Artedius* Girard 1856 and *Orthonopias* Starks and Mann 1911 (Taranets 1941). These early studies united the group using morphological characters that can be found throughout Cottidae (i.e., reduction in scales, reduced number of preopercular spines, three soft pelvic rays; Greeley 1899, Hubbs 1926b, Taranets 1941). Subsequent systematic research added the genera *Phallocottus* Schultz 1938 (Howe and Richardson 1978), *Ruscarius* Jordan and Starks 1895 (Bolin 1944, 1947), and *Leiocottus* Girard 1856 (Bolin 1944, 1947) to the Oligocottinae. The taxonomy of the group underwent frequent reviews (e.g., Greeley 1899, Hubbs 1926b, Jordan and Evermann 1898) until Bolin (1944) consolidated many of the oligocottine genera down to just three: *Clinocottus*, *Oligocottus*, and *Artedius*. Since Bolin's (1944) revision the only taxonomic change within the subfamily was the split of *Artedius* (*sensu* Bolin 1947; 7 spp) into *Artedius* (5 spp) and a resurrected *Ruscarius* (2 spp; Begle 1989). Later studies note the close relationship of oligocottine sculpins but offered limited evidence (i.e., reduction in scales, reduction in preopercular spines) to support the group (Bolin 1944, Bolin 1947, Howe and Richardson 1978). Similarly, studies that apply cladistic methods to oligocottine phylogenetics have yielded only a few putative synapomorphies (see Begle 1989, Strauss 1993).

The phylogeny of Oligocottinae was recently studied with DNA sequence evidence from mitochondrial (cyt b and NADH1) and nuclear (S7 intron 1) gene regions (Ramon and Knope 2008, Knope 2013). Results of those two studies differ from Bolin (1944, 1947) in rejecting the monophyly of *Clinocottus*, and supporting the validity of *Ruscarius*. Notably, results of those studies differ from Bolin (1944, 1947) and from each other in the placement of *Orthonopias triacis* and *Clinocottus acuticeps*. None of these studies included samples of the oligocottine genus *Sigmistes*. *Sigmistes* has been included in only three phylogenetic studies since its description (Hubbs 1926b, Taranets 1941, Howe and Richardson 1978), none of which used explicit phylogenetic methods to infer relationships.

Given the lack of morphological synapomorphies to support the monophyly of Oligocottinae and inconsistent results in recent phylogenetic studies of the subfamily, I

assembled and analyzed an extensive DNA sequence dataset from a broad sample of oligocottine and possible outgroup taxa. The objectives of this study were to: (1) test the monophyly of the subfamily Oligocottinae and each of its constituent genera, (2) test the phylogenetic placement of the oligocottine genus *Sigmistes*, and (3) develop a phylogenetic hypothesis of oligocottine sculpins. Sequence data for this study derived from seven nuclear genome regions and one mitochondrial genome segment. The results of this study show that the genus *Sigmistes* should not be classified as an oligocottine sculpin, the genus *Clinocottus* is polyphyletic, and that the genus *Leiocottus* should be synonymized with *Clinocottus*.

2.3 Methods

2.3.1 Taxonomic sampling

Specimens representing all species of the genera: *Oligocottus*, *Clinocottus*, *Sigmistes*, *Artedius*, *Phallocottus*, *Leiocottus*, and *Orthonopias* were assembled from field and museum collections (Table 2.1). This taxonomic sample includes all species that have been included directly or indirectly within Oligocottinae with the exception of *Ruscarius creaseri* (Hubbs 1926a) and *R. meanyi* Jordan and Starks 1895. Samples from these two species were not available for this study. Nineteen other cottoid species were included in the taxon sample to allow tests of the monophyly of the Oligocottinae. These species were chosen based on phylogenetic relationships hypothesized in previous studies (i.e., Bolin 1947, Yabe 1985, Smith and Wheeler 2004, Knope 2013). The outgroup species represent the genera: *Blepsias* Cuvier 1829, *Chitonotus* Lockington 1879, *Enophrys* Swainson 1839, *Hemilepidotus* Cuvier 1829, *Hexagrammos* Tilesius 1810, *Hemitripterus* Cuvier 1829, *Icelinus* Jordan 1885, *Icelus* Krøyer 1845, *Leptocottus* Girard 1854, *Myoxocephalus* Tilesius 1811, *Percis* Scopoli 1777, *Podothecus* Gill 1861, *Radulinus* Gilbert 1890, *Rhamphocottus* Günther 1874, and *Triglops* Reinhardt 1830.

Sculpins were collected from nearshore and intertidal habitats from 38 localities across Alaska, British Columbia, Washington, and Oregon (Table 2.1). Collections were made from intertidal habitats using dip nets at low tide and from sub-tidal habitats using

SCUBA equipment. Voucher specimen and tissue samples were archived in the fish collections at University of Alaska Museum and the University of Washington. In addition to directed collections, specimens and/or tissue samples were provided by the Alaska Sea Life Center, Mayumi Arimitsu (United States Geological Survey), Milton Love (University of California, Santa Barbara), Marina Ramon (University of Southern California), Scripps Institution of Oceanography, University of Washington Fish Collection, and the University of Kansas. In total, 119 individuals representing 37 species of cottoids were examined in this study.

2.3.2 DNA sequence determinations

Total genomic DNA was extracted from fin and muscle tissue with reagents and protocols from the DNEasy Blood and Tissue Kit (Qiagen Corp.). DNA fragments from eight molecular loci (Table 2.2): one mitochondrial protein-coding locus (Cytochrome *c* oxidase, COI), two nuclear introns [exon-primed intron crossing (EPIC) locus 1777E10 and EPIC locus 4174E20] and five protein-coding nuclear loci [early growth response protein 1 (EGR1); mixed-lineage leukemia (MLL); patched domain-containing protein 1 (ptchd1); Rhodopsin; and Sushi, von Willebrand factor type A, and pentraxin domain-containing 1 (SVEP)] were amplified by targeted polymerase chain reactions (PCR). Standard reagent concentrations (1X Buffer, 0.8mM dNTP, 1-2mM Mg^{++}, 0.4µM F/R primer, 0.025 U/µl *Taq* polymerase, and 1 µl of DNA template of variable concentration per 25 ul reaction) were used in all reactions. With the exception of SVEP, thermalcycler profiles for each reaction were adapted from the primer sources for each locus (see Table 2.2), with minor adjustments to annealing temperature and/or extension time. A nested PCR strategy was used to generate amplicons of ptchd1 and SVEP suitable for sequence determination. For SVEP, novel primers were designed for the nested reaction (see Table 2.2) and, for this second reaction, the thermal cycler conditions were as follows: initial denaturation at 94°C for 90 seconds (s); 40 cycles of 94°C denaturation for 30s, 65°C annealing for 30s, 72°C extension for 45s; and final extension at 72°C for 4 minutes.

Amplicons were purified and sequenced in both directions by Sanger sequencing at the University of Washington High-Throughput Genomics Unit. Sequences were

trimmed, visually checked for quality, and assembled into forward-reverse contiguous sequences using CodonCode Aligner Software (CodonCode Corp.) Multiple sequence alignments (MSAs) for each locus were generated in ClustalW (Larkin et al. 2007). Alignments were trimmed and reading frame established using Se-Al (Rambaut 2002). MSAs for all loci were concatenated using Mesquite (Maddison and Maddison 2011).

2.3.3 Phylogenetic inference

To assess the possible effects of analysis-specific inference artifacts, multiple phylogenetic approaches were used. Phylogenetic relationships were estimated by analyses of the concatenated dataset using Maximum Likelihood (ML), Bayesian (B), and Maximum Parsimony (MP) optimality criteria. ML and B inference incorporate statistical models of sequence evolution that include nucleotide substitution rates, base composition frequency, proportion of invariant sites, and rate variation among sites. Using these parameters, the models can take into account the potential for unobserved nucleotide mutations (e.g., an A mutating into a T, then mutating back to an A) and incorporate branch length data (i.e., the rate of nucleotide substitutions) when searching through trees (Strimmer and von Haeseler 2009). Assuming the user-specified model, ML searches for the tree topology that maximizes the likelihood of the observed data (i.e., the concatenated MSA dataset) (Schmidt and von Haeseler 2009). Bayesian inference generates the posterior probability of a phylogeny given the data, using likelihood and user-defined prior probability distributions (Ronquist et al. 2009). MP does not allow the user to specify the model of sequence evolution. Instead, MP searches for the phylogenetic tree that minimizes the number of necessary evolutionary events (in this case, nucleotide substitutions) in the dataset (Swofford and Sullivan 2009). MP does not incorporate branch length data when searching through trees, but rather assigns branch lengths after the tree has been assembled, based on the number of state changes between nodes of the final tree. Discrepancies and similarities between the results of the analyses were used to evaluate the confidence of relationships inferred in the B analysis, the results of which served as the primary phylogeny. In particular, support from all three

analyses was taken to be an indication of a strong signal in the dataset for a given relationship.

Maximum likelihood analysis of the concatenated dataset was conducted with RaxML v. 7.3.0 (Stamatakis 2006) using the rapid bootstrapping algorithm (Stamatakis et al. 2008). The dataset was partitioned by locus (e.g., COI, EGR1, etc.) and the general time reversible (GTR) model of molecular evolution with a four-category gamma distribution of rate variation and invariable sites was applied to each data partition. A bootstrap analysis with 5,000 iterations was performed to assess the strength of different components of the phylogenetic inference.

The best fitting model of molecular evolution for each locus was identified using the Akaike information criterion (AIC; Akaike 1973, Posada and Buckley 2004) with the model comparison routines implemented in MrModeltest (Nylander 2004). Essentially, AIC values correspond to the amount of information lost when using a given model of molecular evolution, compared to the way in which the molecules are "truly" evolving (Kullback and Leibler 1951, Posada and Buckley 2004). The model with the lowest AIC value, therefore, offers the best fit to the data.

Bayesian analysis of the concatenated dataset was conducted in MrBayes v. 3.2.0 (Ronquist et al. 2012). The dataset was partitioned by locus and each partition was assigned the best-fitting model structure as determined in MrModeltest. Character state frequencies, substitution rates, gamma shape parameter, and proportion of invariable sites were unlinked across partitions. A 50% consensus tree was generated from six independent runs of 20 million generations, sampled every 5,000 generations, with the first 25% discarded as burn-in.

For analysis using parsimony, the number of samples and loci proved to be computationally prohibitive, so a single chimeric sequence was generated for each species from the most common allele/haplotype among individuals of that species at each locus. This treatment reduced the number of samples to equal the number of species and made a parsimony analysis computationally feasible. The parsimony analysis of the reduced dataset was conducted in PAUP* 4.0b10 (Swofford 2003). Starting trees were

obtained using stepwise addition with additional sequences added randomly. Branch swapping was conducted with the tree-bisection-reconnection (TBR) algorithm. Bootstrap values and a 50% majority-rule consensus tree were generated with 1000 bootstrap iterations using the same heuristic search strategy. To evaluate the effect of the data reduction strategy on inference, the reduced MSA was analyzed by maximum likelihood and Bayesian inference to compare the topology of the trees generated from reduced and full datasets under similar analytical frameworks.

The phylogeny was tested for the presence of destabilizing "rogue" taxa using RogueNaRok (Aberer et al. 2013). Any individuals that failed to find consistent placement among pseudo-replications were flagged as problematic (Aberer et al. 2013), removed from the alignment, and phylogeny re-inferred from the reduced dataset.

2.3.4 Alternative coding and data permutations

To evaluate the effect of alternative partition and sequence coding schemes, alternative schemes were tested using maximum likelihood and Bayesian inference. These alternative coding/partitioning schemes included: treating the dataset as a single partition, partitioning by gene and codon position [i.e., Gene (1_N, 2_N, 3_N)], partitioning by gene with the third codon position sites coded as only purines/pyrimidines [i.e., Gene ($1_N2_N3_{RY}$)], and partition by gene with deletion of the third codon position sites [i.e., Gene (1_N2_N)]. These latter two permutations of the dataset were used to test for substitution saturation of the third codon position site in the protein-coding loci. The optimal partitioning scheme was also tested using PartitionFinder (Lanfear et al. 2012) under the Bayesian information criterion (BIC). For this analysis, each protein-coding region was broken up by codon position (cp) so that, for example, COI was broken into three loci: COI _cp1, COI _cp2, and COI _cp3. Here again discrepancies and similarities between the results of the analyses were used to evaluate the confidence of relationships inferred in the primary B analysis, especially to identify strong signals in the dataset.

2.4 Results

2.4.1 Sequences

In order to account for length variability among sequences, the MSAs were trimmed to a common length for each locus (Table 2.3). These MSAs were concatenated and the resulting dataset contained a total of 4696 aligned nucleotide sites, of which 1037 were variable and, of those, 368 were parsimony-informative. Table 2.3 shows the divergence statistics of each locus.

For each locus, the following models of molecular evolution have the lowest AIC values and therefore represent the best fit: EPIC locus 1777E4 and SVEP best fit the General Time Reversible (GTR) model (Tavaré 1986) with among site rate variation (ASRV); COI, ptchd1, and Rhodopsin best fit the GTR model with ASRV and invariable sites; EPIC locus 4174E20 and MLL best fit the Hasegawa-Kishino-Yano (HKY; Hasegawa et al. 1985) model with ASRV; and EGR1 best fit the HKY model with ASRV and invariable sites. These models were used in the Bayesian analyses of the concatenated dataset.

The analyses conducted in PartionFinder showed that the optimal partitioning scheme consists of ten partitions, with the third codon position of each protein-coding region often as a distinct partition (Table 2.4). This partitioning scheme was used for an additional Bayesian analysis of the dataset, run for five million generations but with otherwise identical parameters to the analysis partitioned by locus.

2.4.2 Phylogenetic relationships

Likelihood and Bayesian inferred tree topologies were largely congruent. Figure 2.1 shows the 50% majority-rule consensus tree produced by the Bayesian analysis, with both Bayesian posterior probabilities (Bpp; scale of 0.00 – 1.00) and bootstrap support (bs; scale of 0 – 100%) values indicated at each node. The only difference between the Bayesian and ML topologies is the placement of *Phallocottus obtusus*, which is placed as sister to *Sigmistes smithi* in the Bayesian inference and as sister to a monophyletic *Sigmistes* under maximum likelihood, but in both cases indices of support for the

conflicting relationships are weak (<0.55 and <0.55%). Both analyses show strong support for a clade consisting of the members of the oligocottine genera *Clinocottus, Orthonopias, Artedius*, *Oligocottus*, and *Leiocottus* (clade A in Fig. 2.1). Neither inference places within that clade the genera *Sigmistes* or *Phallocottus*. Rather, these two genera are allied to the genus *Icelus* with strong and unanimous support across all analyses (1.00 Bpp and 100% bs).

Within clade A, there are two primary lineages: *Clinocottus acuticeps* + the genus *Artedius* (clade B in Fig. 2.1), and a group consisting of all the remaining species of clade A (clade C in Fig. 2.1). Within clade B, *A. corallinus* is sister to *A. lateralis* with unanimous support; *A. notospilotus* is sister to *A. fenestralis* with high support (1.00 Bpp and 98% bs); these two groups are most closely related to one another; and this larger group is sister to *A. harringtoni*. This larger group corresponds to the genus *Artedius* and is well supported (1.00 Bpp and 98% bs). *Artedius* and *Clinocottus acuticeps* are placed as sister taxa but the relationship is weakly supported (0.58 Bpp and <50% bs). Within the *Artedius* clade, the sister relationship of the *A. fenestralis* + *A. notospilotus* clade with the *A. corallinus* + *A. lateralis* clade is only moderately supported (0.65 Bpp and 78% bs; Fig. 2.1).

Within clade C, there are two primary lineages: *L. hirundo* + *C. analis*, which was supported unanimously (clade D in Fig. 2.1), and a weakly supported (0.640 Bpp and 53% bs) clade containing all remaining taxa (clade E in Fig. 2.1). Clade E is split into two well-supported groups: the genus *Oligocottus* and a clade containing *Orthopias triacis*, *C. recalvus*, *C. globiceps*, and *C. embryum* (clade F in Fig. 2.1). Within clade F, all relationships were well resolved with high support indices (Fig. 2.1): *C. recalvus* is sister to *C. globiceps*; this group is sister to *C. embryum*; and this larger group (clade G in Fig. 2.1) is sister to *O. triacis*. Within *Oligocottus*, all relationships were well resolved with high support indices (Fig. 2.1): *O. rubellio* is most closely related to *O. snyderi*; this group is sister to *O. maculosus*; and this larger group (clade H in Fig. 2.1), is sister to *O. rimensis*.

Topologies from likelihood and Bayesian analyses of the reduced dataset were congruent with those produced using the full dataset, only differing in the placement of *C. acuticeps*. In the full dataset *C. acuticeps* was allied to the *Artedius* clade, while in the reduced dataset *C. acuticeps* was placed in a polytomy with *Artedius* and clade C.

The majority-rule consensus tree produced by the parsimony analysis had a similar topology to the likelihood and Bayesian phylogenies but with some notable differences (Fig. 2.2). Like the other inferences, the parsimony tree showed strong support for clade A (100% bs). Like the BI and ML phylogenies (Fig. 2.1), clade A did not include *Sigmistes* or *Phallocottus*. Instead, these genera were again allied to *Icelus spiniger* with high support (95% bs). The MP phylogeny also showed strong to moderate support for monophyly of: the genus *Artedius* (87% bs); clade G (89% bs); clade D (86%), and clade H (77%). Likewise, the relationships of the remaining taxa in the parsimony tree were very similar to their likelihood and Bayesian counterparts, but with the following exceptions: where the ML and BI phylogenies showed a monophyletic *Oligocottus*, and allied *Orthonopias triacis* with clade G, the parsimony analysis did not provide a resolution for the placement of *Oligocottus rimensis* or *Orthonopias triacis*. It also did not resolve the relationships of clades D, G, and H. Rather, in the parsimony tree those groups form a polytomy that also includes *O. rimensis* and *O. triacis*.

RogueNaRock testing suggested the removal of five individuals from the alignment. However, these rogue individuals were from different species represented by multiple individuals, as a result no species was excluded from the alignment after pruning. Inter-species relationships before pruning and after pruning were unchanged. Pruning had minor changes in some support values (i.e., <10%).

All alternative coding and partitioning schemes produced phylogenies with similar topologies to those produced by the initial BI and ML analyses. The two coding schemes that produced the greatest deviations were those that affected third codon position sites by either coding them as RY or deleting them. RY coding of third codon position sites affected the tree topology by changing the relationship of *C. acuticeps* from a weakly supported sister-group of *Artedius* to a weakly supported (i.e. <60% bs) sister of

the Oligocottini, collapsing the sister relationship of *Oligocottus* and clade F, and by collapsing the sister relationship of *O. triacis* with clade G. Additionally, this coding scheme showed strong support (94% bootstrap) for the clade that contains *A. corallinus*, *A. lateralis*, *A. notospilotus*, and *A. fenestralis*, compared to the low to moderate support (0.650 Bpp and 78% bs) for this clade in the unaltered BI and ML phylogenies (Fig. 2.1). Deleting the third codon position sites produced similar results with the only notable differences being: a weakly supported (0.540 Bpp) sister-group relationship of *O. triacis* and clade G; collapse of the sister-group relationship between *O. rimensis* and the clade H to form a polytomy between *O. rimensis*, *O. maculosus*, and the *O. rubellio* + *O. snyderi* clade; moderate support for a sister-group relationship between *Oligocottus* and the members of clade G; a collapse of the relationship between *A. lateralis* and *A. corallinus*, nesting *A. corallin*us within *A. lateralis*; and a collapse of the *A. notospilotus* + *A. fenestralis* clade; and strong support (99% bs) for the clade containing *A. lateralis*, *A. corallinus*, *A. notospilotus*, and *A. fenestralis*. It should be noted that the phylogeny produced after the complete deletion of third codon position sites was the only instance where monophyly of clade H was not supported.

2.4.3 Phylogenetic hypothesis

Figure 2.3 shows a cladogram depicting the most stable and well-supported relationships based on the results described above. The purpose of this phylogeny is to highlight clades supported by a strong phylogenetic signal in the sequence data as evidenced by the consistent appearance and strong support of a given clade in multiple analyses and permutations of the data. Relationships not unanimously supported by the Bayesian inference, maximum likelihood, and parsimony analyses were collapsed if the support values were low in the initial ML and BI analyses (i.e., >0.65 Bpp and >65% bs) and in those using alternative phylogenetic approaches (i.e., clade E, clade B, and the relationship of *Phallocottus obtusus*, *Sigmistes caulias*, and *S. smithi*). The sister-group relationship of the *Artedius fenestralis* + *A. notospilotus* clade with the *A. lateralis* + *A. corallinus* clade was not supported by the parsimony analysis (Fig. 2.2) and was only moderately supported in the initial analyses (Fig. 2.1). However, a clade containing these

four species was well supported in the phylogenies produced by alternative coding of the third codon position (94-99% support values), and has been proposed by some previous studies (i.e. Bolin 1944, Bolin 1947, Ramon and Knope 2008), though others (i.e., Begle 1989, Knope 2013) came to different conclusions. Several clades, however, were supported by the primary and all alternative analyses: *Sigmistes* + *Phallocottus*, together allied with *Icelus spiniger*; clade A, *Artedius*, clade C, clade G, clade D; the sister-group relationship of *O. snyderi* with *O. rubellio*; and the monophyly of *A. lateralis* + *A. corallinus*.

2.5 Discussion

2.5.1 Oligocottine monophyly

Since the subfamily Oligocottinae was first delineated, its monophyly has been examined in several studies (i.e., Strauss 1993, Ramon and Knope 2008, Knope 2013). Although these studies did not examine all genera assigned to the Oligocottinae, they support a close relationship between *Oligocottus*, *Clinocottus*, *Orthonopias*, and *Artedius*, including the monotypic genus *Leiocottus* in this group, in agreement with earlier work (e.g., Bolin 1944, 1947). Broader systematic studies of cottoid relationships (i.e., Yabe 1985, Jackson 2003, Smith and Wheeler 2004) have examined only single representatives of some of the oligocottine genera, which, aside from *Artedius* (see Begle 1989), had themselves not been systematically tested for monophyly until recently (i.e., Ramon and Knope 2008, Knope 2013).

The evidence presented here provides strong support for a monophyletic group consisting of the genera: *Clinocottus*, *Oligocottus*, *Artedius*, *Leiocottus*, and *Orthonopias* (Clade A in Fig. 2.1 and Fig. 2.2). This grouping was present and strongly supported in the analyses of every permutation of the data and all methods of phylogenetic inference (Fig. 2.3). This grouping is in agreement with early evolutionary hypotheses (i.e., Bolin 1944, 1947), the morphology-based cladistic analysis of the genera (Strauss 1993), and recent molecular-based analyses of many of the oligocottine species (Ramon and Knope 2008, Knope 2013). Significantly, no analyses conducted in this study placed the genus

Sigmistes in clade A. Instead, every analysis allied *Sigmistes* with the monotypic genus *Phallocottus*, and this clade was allied with *Icelus spiniger* with very high support values (Fig. 2.3). This finding contradicts previous work, which had unanimously allied *Sigmistes* with *Clinocottus* (Hubbs 1926b, Taranets 1941, Howe and Richardson 1978). However, it should be noted that the present study represents the first analysis of the phylogenetic placement of either *Sigmistes* or *Phallocottus* using formalized methods of phylogenetic inference.

The traits that have been proposed to delineate Oligocottinae (e.g., soft pelvic rays, simple preopercular spines; see Hubbs 1926b, Taranets 1941) are found throughout the cottoid suborder (see descriptions in Bolin 1944). Studies that have analyzed the morphology of oligocottine species in a cladistic framework have either failed to resolve their relationship (e.g., Jackson 2003) or been so limited in taxon-sampling that the results are difficult to interpret in either a broad phylogenetic sense or as being generally applicable to all oligocottines (Washington 1986, Begle 1989, Strauss 1993). Given the results presented here and the absence of relevant contradictory evidence in the literature, I propose to delineate the Oligocottinae to include only the members of clade A, thus the genus *Sigmistes* should removed from the subfamily (Table 2.5).

2.5.2 Inter-generic relationships

Outside of Oligocottinae, as defined above, lies the *Sigmistes* + *Phallocottus* clade. These genera were grouped together and as a sister-group to *Icelus spiniger* in every analysis conducted in this study. Within the *Sigmistes* + *Phallocottus* clade, however, there was great discrepancy on the placement of *Phallocottus*; some analyses placed it as sister to *Sigmistes* while others nested it within *Sigmistes* as sister to *S. smithi*. Given the lack of clear consensus and the low support values for either of the two placements of *Phallocottus*, the relationship of the constituent species of the two genera could not be confidently resolved, so they were collapsed to form a polytomy (Fig. 2.3). Regardless, the species of these genera together form a stable and well-supported monophyletic group.

Within Oligocottinae, there was unambiguous support for division of the subfamily into three primary lineages: *Artedius*, clade C (hereafter referred to as the tribe Oligocottini *sensu* Hubbs 1926b, modified from Taranets 1941), and *Clinocottus acuticeps*. Relationships between these three lineages vary between analyses. Thus, given the available evidence, these three lines are best arranged in an unresolved trichotomy at the base of the oligocottine clade.

Within the tribe Oligocottini there are three unambiguously supported groups: the genus *Oligocottus,* clade D, and clade F. Clade F contains clade G, corresponding to the subgenus *Blennicottus* Gill 1861 *sensu* Bolin 1944, and *O. triacis*. These two groups share very little apparent morphological similarity aside from an anteriorly placed vent (Bolin 1944). However, given the lack of consensus and poor resolution of phylogenetic relationships among oligocottine sculpins presented in previous morphological studies (Bolin 1944, Yabe 1985, Begle 1989, Strauss 1993, Jackson 2003), any claim of shared ancestry or lack thereof based on morphology must be considered speculative at best. Therefore, given the support of a close relationship between *O. triacis* and *Blennicottus* found in this study, I conclude that the physical differences (notably, presence of scales and number of preopercular spines) do not necessarily preclude a close relationship of the two taxa. Further study of morphology may provide insight into the apparent discrepancy between genotype and phenotype in this group.

2.5.3 Monophyly of oligocottine genera

2.5.3.1 *Artedius* Girard 1856

My results show strong support for monophyly of the genus *Artedius* and moderate support for the intra-generic relationships suggested in previous morphological (i.e., Bolin 1947) and molecular (i.e., Ramon and Knope 2008) studies (Fig. 2.1, Fig. 2.3).

2.5.3.2 *Clinocottus* Gill 1861

The results reported here show that *Clinocottus* in its current composition does not represent a natural group, but rather an artificial assemblage of three distinct and

distantly related lineages (Fig. 2.1, Fig. 2.3). The first line includes only *C. acuticeps*. Its phylogenetic placement is at the base of the oligocottine tree as part of a polytomy with *Artedius* and Oligocottini. I conclude that *C. acuticeps* likely represents an early offshoot within Oligocottinae, and future studies using additional lines of evidence (i.e., ontogenetics) may clarify the relationship of this species to other oligocottine lineages.

The second lineage within *Clinocottus* is the *C. analis* lineage. Every permutation and analysis conducted in this study shows a highly supported clade consisting only of *C. analis* + *Leiocottus hirundo*. This relationship was also shown in other DNA-based phylogenies (i.e., Ramon and Knope 2008 and Knope 2013). No cladistic, morphology-based study has examined both *C. analis* and *L. hirundo*. However, Bolin (1944, 1947) considered *L. hirundo* to be allied to the genus *Clinocottus* and noted differences in the attachment of the gill membrane as the only notable distinction between the genera. Shared characters between the two genera include the "structure of the preopercular spine," "advanced anus," and "blunt" genital papilla (Bolin 1947). The latter two features are some of the most notable distinguishing features of *Clinocottus*, as described in Bolin (1944). I therefore conclude that given the overwhelming DNA-based support, the shared morphological traits between *Clinocottus* and *L. hirundo*, and the lack of tested, morphological evidence distinguishing *L. hirundo* from *Clinocottus*, *L. hirundo* should be placed in the genus *Clinocottus* (Table 2.5).

The final lineage within *Clinocottus* is the subgenus *Blennicottus*. This group contains *C. recalvus*, *C. globiceps*, and *C. embryum*. Morphologically, these species are united with each other and differentiated from other members of *Clinocottus* by the "deep" and "heavy" caudal peduncle and a comb of cirri at each anterior pore of the lateral line (Bolin 1944). *C. globiceps* and *C. recalvus* have been closely allied throughout their taxonomic history, and were in fact believed to be subspecies for a time (see Greeley 1899). It comes as no surprise then that within the subgenus *Blennicottus*, *C. globiceps* and *C. recalvus* are most closely related, with *C. embryum* as sister to their clade. This grouping and structure are shown with high support in all permutations and analyses of my dataset with the exception of the treatment of removing the third codon

position from the alignment, where the overall grouping remains with high support but the relationship of the three species is unresolved. Recent, DNA-based studies have shown the same relationship of *C. embryum*, *C. globiceps*, and *C. recalvus* (Ramon and Knope 2008, Knope 2013).

The larval-character based study by Washington (1986) and the follow up study by Strauss (1993), incorporating Washington's character matrix with that of Begle (1989), placed *C. embryum* in a separate clade from *C. recalvus* and *C. globiceps.* However, those results were not generated using formalized analyses or suffered from methodological flaws (as described above). Given the strong support in my analyses, coupled with the support of other DNA-based studies, synapomorphies for the group, and the relatively few characters used in the only dissenting studies (i.e., Washington 1986 and Strauss 1993), I conclude that the subgenus *Blennicottus* (*sensu* Bolin 1944) is a valid taxonomic grouping, distinct from other members of *Clinocottus*.

2.5.3.3 *Leiocottus* Girard 1856

Leiocottus hirundo forms a well-supported clade with *C. analis* in every analysis conducted in this study (Fig. 2.3). Considering this evidence, the morphological similarities between *Leiocottus* and *Clinocottus* reported in previous studies (i.e., Bolin 1944, 1947), and the similar findings of other DNA-based studies (i.e., Ramon and Knope 2008, Knope 2013), I conclude that *L. hirundo* should be placed in the genus *Clinocottus* where it and *C. analis* would make up the subgenus *Clinocottus*, (modified from Bolin 1944, 1947; see Fig. 2.3, Table 2.5).

2.5.3.4 *Oligocottus* Girard 1856

Members of *Oligocottus* form a well-supported clade in the phylogenies produced by the primary ML and B phylogenies (Fig. 2.1), as well as the phylogeny produced by coding the third codon position sites as R/Y. *Oligocottus rimensis* is morphologically distinct from the rest of *Oligocottus* in that its body is almost completely covered in prickles, and the upper preopercular spine is simple, but is united with other members of the genus by the general modification of the anterior anal fin rays and the placement of

the vent with respect to the anal fin (see Bolin 1944, 1947). The rest of the members of *Oligocottus* (*O. maculosus*, *O. snyderi*, and *O. rubellio)* make up clade H, (subgenus *Oligocottus* in Bolin 1944, 1947) and were well supported in my analyses and united by several morphological similarities: complete loss of all scales but those on the lateral line, bifurcation or trifurcation of the upper preopercular spine, and a simple, elongated genital papilla in males (see Bolin 1944, 1947). Within this group, *O. snyderi* and *O. rubellio* are most closely related, and their sister-relationship was recovered in all analyses. The close relationship of these two species is supported morphologically by the modification of the anterior-most anal fin ray into an elongated, prehensile organ (used by *O. snyderi* to grasp females during copulation; Morris 1956), and by the abundance and distribution of multifid/palmate cirri across their head and body (Bolin 1944, 1947). I conclude that *Oligocottus*, in its present form, forms a monophyletic, well-defined, and strongly supported clade.

2.5.3.5 *Orthonopias* Starks and Mann 1911

Perhaps most confounding of any lineage within Oligocottinae is the monotypic *Orthonopias*. Previous morphological studies have allied this species with *Artedius* (i.e., Taranets 1941, Bolin 1944, Bolin 1947) or placed it completely outside of Oligocottinae (i.e., Begle 1989). Indeed, *O. triacis* possesses an unusual mixture of primitive and derived traits (*sensu* Bolin 1944, 1947) compared to other oligocottine species. For example, *O. triacis* possesses four distinct preopercular spines while in all other oligocottine sculpins only the uppermost preopercular spine is distinct and the lower three are either reduced to small nubs or are completely obsolete. *O. triacis* is also the only oligocottine species to have both an advanced anus and an *Artedius*-type dorsal scale band. Additionally, *O. triacis* has a unique morphology of the pelvic fins that is sexually dimorphic (see Bolin 1944, 1947).

The unique suite of morphological characters found in *Orthonopias triacis* has perhaps contributed to the lack of consensus among attempts to infer its phylogenetic placement using comparative morphology (i.e., Taranets 1941, Bolin 1944, Bolin 1947, Begle 1989, Jackson 2003). There is, however, strong support on the molecular level for

the phylogenetic placement of *O. triacis* within clade C, and a strong association between *O. triacis* and the subgenus *Blennicottus* (Fig. 2.1).

2.5.3.6 *Phallocottus* Schultz 1938

The monotypic *Phallocottus* is presently known only from a few locations in the Aleutian archipelago. This genus has received little scientific study and, prior to this investigation, the phylogenetic placement of *Phallocottus* had never been examined. The initial description of *P. obtusus* asserted that it was "most closely related to the Oligocottinae of Hubbs, 1926," especially comparing several morphological features (e.g., the arched lateral line) with those found in the oligocottine (*sensu* Hubbs 1926b) genus *Sigmistes* (Schultz 1938). *Phallocottus obtusus* was considered distinct from other oligocottine sculpins based primarily on its rounded preopercular spine, lack of palatine teeth, and obscured nasal spines (Schultz 1938). The only other mention of *Phallocottus* in an evolutionary context is found in an unpublished study of meristic characteristics of NE Pacific sculpins, which agreed with the conclusions of Schultz (1938) that *Phallocottus* was most closely related to *Sigmistes* (Howe and Richardson 1978).

All of the results of the present study show strong support for a clade containing *P. obtusus* and both species of *Sigmistes*. However, because the results do not resolve the relationship of these three species, I consider an unresolved 'soft' polytomy consisting of *P. obtusus*, *S. smithi*, and *S. caulias* as the best phylogenetic estimate available at present. The morphological features uniting *Phallocottus* and *Sigmistes* are a lack of scales and a strong arch in the lateral line above the pectoral fins (see Jordan and Evermann 1898, Schultz 1938, Howe and Richardson 1978). Given the consistent and overwhelming support of a monophyletic relationship of the members of these two genera in all of my analyses, combined with the morphological similarities and historical affinities, I conclude that *Phallocottus* forms a monophyletic group with the members of the genus *Sigmistes*.

2.5.3.7 *Sigmistes* Rutter 1898

Sigmistes was allied with the modern genus *Clinocottus* in the earliest systematic classifications of Oligocottinae (Hubbs 1926b, Taranets 1941). Prior to that, *Sigmistes* was allied to *C. acuticeps* and *C. embryum* (Jordan and Evermann 1898). Indeed, there are several morphological similarities between *Sigmistes* and members of *Clinocottus* (e.g., a lack of scales, advanced anus, enlarged genital papilla in males). However, none of those similarities has been tested in any kind of systematic analysis. In fact, the only other mention of the evolutionary relationships of *Sigmistes* was in an unpublished meristic study, which asserted that *Sigmistes* is closely related to *Phallocottus*, and the two genera together are most closely related to the "*Oligocottus*-*Clinocottus* group," especially *Clinocottus* (Howe and Richardson 1978), but gives no indication of the evidence supporting such a grouping.

The sequence data presented here overwhelmingly support a close relationship between *Sigmistes* and *Phallocottus* but ally them to *Icelus spiniger* rather than *Clinocottus*. Furthermore, my results never place the *Sigmistes* + *Phallocottus* clade within, or even sister to the Oligocottinae, as defined in this study

The relationship between *Sigmistes* and *Phallocottus*, or rather, the validity of *Phallocottus*, is unclear. As was discussed above, the relationship of *S. smithi*, *S. caulias*, and *P. obtusus* is inconsistent and weakly supported across our various analyses, making a confident resolution impossible. Rather, I place the three species in a polytomy.

As in the case of *Phallocottus*, the present study represents the first test of the classification and phylogenetic placement of *Sigmistes*. Given the strong support of a close relationship between *Sigmistes* and *Phallocottus*, the morphological similarities between the two genera (see above discussion of *Phallocottus*), I conclude that *Phallocottus* and *Sigmistes* form a well-supported, monophyletic group. Additionally, I conclude that neither *Sigmistes* nor *Phallocottus* should be classified as oligocottine sculpins, despite some of the superficial morphological similarities (Table 2.5).

2.6 Conclusions

The subfamily Oligocottinae should be revised to include only the genera: *Oligocottus*, *Clinocottus*, *Orthonopias*, and *Artedius* (Table 2.5). The genus *Sigmistes* should not be grouped in this subfamily, despite the superficial morphological similarities is shares with some members of the oligocottine genus *Clinocottus*. Rather, *Sigmistes* and the genus *Phallocottus* form a monophyletic clade, closely related to the genus *Icelus.*

Artedius and *Oligocottus* are both consistently supported as monophyletic genera within Oligocottinae. The genus *Clinocottus* is polyphyletic as currently defined as it comprises three distinct lineages that have closer affinities to other oligocottine groups than they do to one another. The three independent lines of *Clinocottus* are the subgenus *Blennicottus*, the subgenus *Clinocottus* Gill 1861, and *Clinocottus acuticeps*. The monotypic genus *Leiocottus* is clearly part of the *Clinocottus* lineage, and its distinction at the generic level carries no benefit of morphological clarity, especially given the morphological diversity within *Clinocottus* (see descriptions in Bolin 1944). I therefore conclude that it should be synonymized and its sole constituent species should be renamed *Clinocottus hirundo* and classified together with *C. analis* as a member of the subgenus *Clinocottus* (Table 2.5).

The morphologically distinct and monotypic genus *Orthonopias* is in need of further study to resolve its phylogenetic placement with confidence. Regardless, *Orthonopias* is not closely related to *Artedius*, as previously proposed. Rather, it represents a morphologically distinct lineage among the *Clinocottus* and *Oligocottus* lineages, with strong molecular support for a close relationship with the subgenus *Blennicottus*.

The groups receiving consistent and strong support in this study represent three evolutionary lines within Oligocottinae: the genus *Artedius*, the tribe Oligocottini, and *Clinocottus acuticeps* (Table 2.5). The Oligocottini form a polytomy containing three distinct groups: the subgenus *Clinocottus*, the genus *Oligocottus*, and a clade containing *O. triacis* plus the subgenus *Blennicottus*.

These revisions to the classification and taxonomy of oligocottine sculpins reflect our best understanding of how the species are related to one another. The phylogeny presented here improves on prior hypotheses that were based on limited data and/or subjective methods. Understanding the phylogeny of a given group facilitates and is prerequisite to accurate studies of biological diversity and macroevolution in said group.

2.7 Future work

The genus *Ruscarius* was not included in the original delineations of Oligocottinae, but was synonymized into the oligocottine genus *Artedius* by a later study (Bolin 1944). This relationship was revised, however, by the first cladistic analysis of *Artedius*, which placed *Ruscarius* as sister to the monotypic genus *Chitonotus* (Begle 1989). Recent, DNA-based analyses, however, have placed *Ruscarius* as sister to the Oligocottinae (as delineated here). As previously mentioned, tissue samples of either species of *Ruscarius* were unavailable at the time of this study. Should such samples become available in the future, it would be interesting to test the conclusions of previous studies with regard to the placement of *Ruscarius*.

Additionally, the relationship of the *Sigmistes* + *Phallocottus* clade with *Icelus spiniger* is extremely well supported in this study. Future studies should include additional species of *Icelus* in order to more completely explore the relationship of *Icelus* with *Sigmistes* and *Phallocottus*.

2.8 Acknowledgements

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(Scripps Institution of Oceanography), Mayumi Arimitsu (United States Geological Survey), Milton Love (University of California Santa Barbara), and Marina Ramon (University of Southern California). W. Leo Smith (University of Kansas) graciously shared his knowledge of cottoid systematics. I would also like to thank Greg Jensen (University of Washington) for his mentorship in identification, collection, and appreciation of sculpins, as well as Matthew A. Campbell (University of Alaska) for the frequent and generous donations of his considerable technical expertise. A special thanks to Jeff Williams (USFWS) and Capt. Billy Pepper (USFWS) for the opportunity to travel aboard the R/V Tiglax and I thank them, Lisa Spitler (USFWS), and the crew of the R/V Tiglax for the assistance and support they provided during my collection activities in the Aleutian Islands.

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2.9 Figures

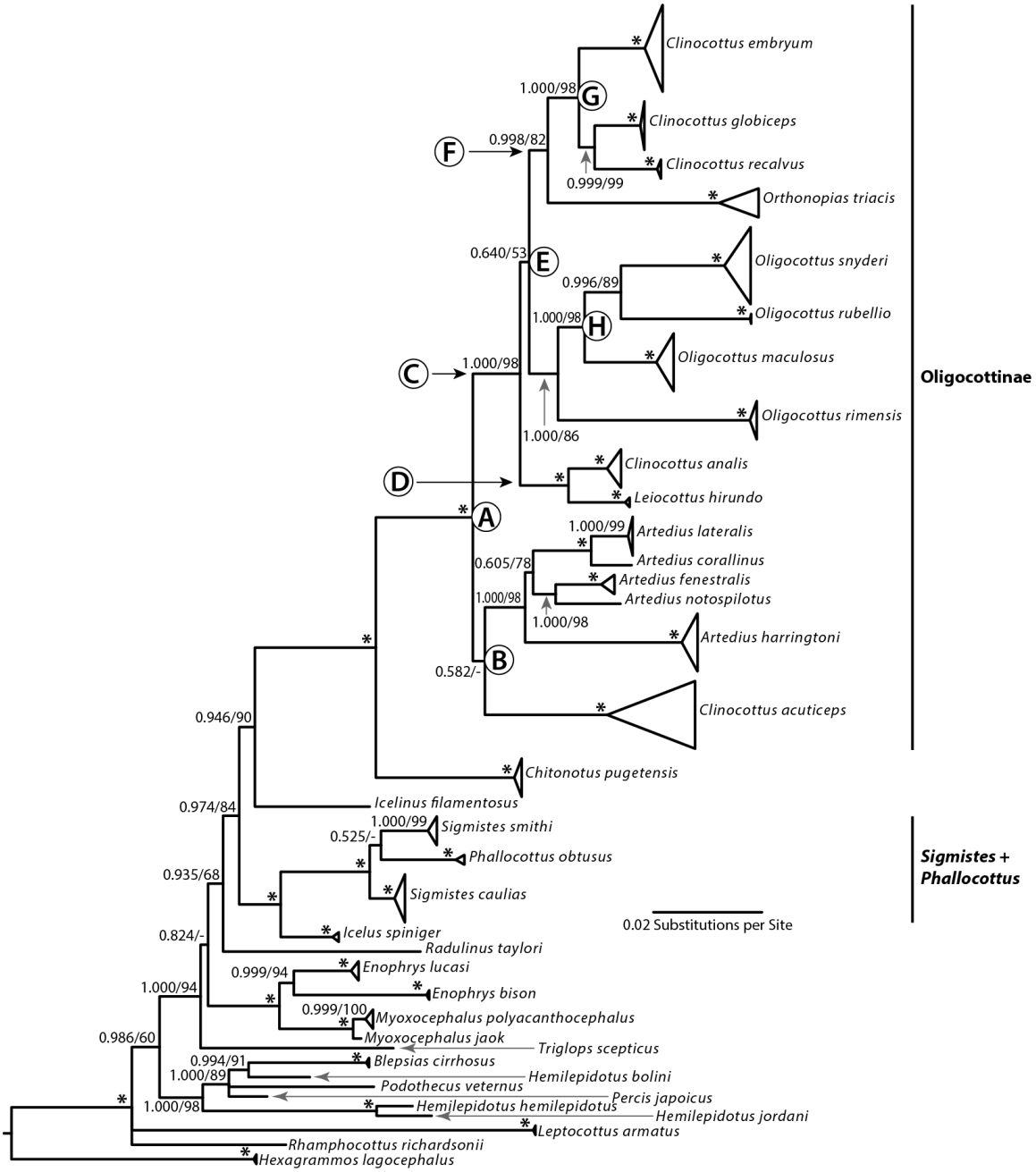

Figure 2.1: Bayesian reconstruction of oligocottine relationships. Phylogeny of Oligocottinae and outgroup taxa was inferred using Bayesian inference of eight concatenated molecular loci, partitioned by locus. Support values are indicated at each node, with Bayesian posterior probabilities followed by bootstrap values generated from

5000 iterations of a maximum likelihood analysis of the dataset using identical partitions. Asterisks indicate 100% support for a given node in both maximum likelihood and Bayesian inference. Dash mark in place of a support value indicates less than 50% support by an analysis for a given node. In every case where multiple individuals of given species were included in the analyses, all members of the same species grouped together as a well-supported clade. For clarity, these clades were collapsed to form a single node, with a delta symbol representing the combined tips. The size of the delta symbol correlates to the amount of diversity within the collapsed clade. Where necessary, arrows are used to indicate the node or tip to which a set of support values or a taxon label corresponds.

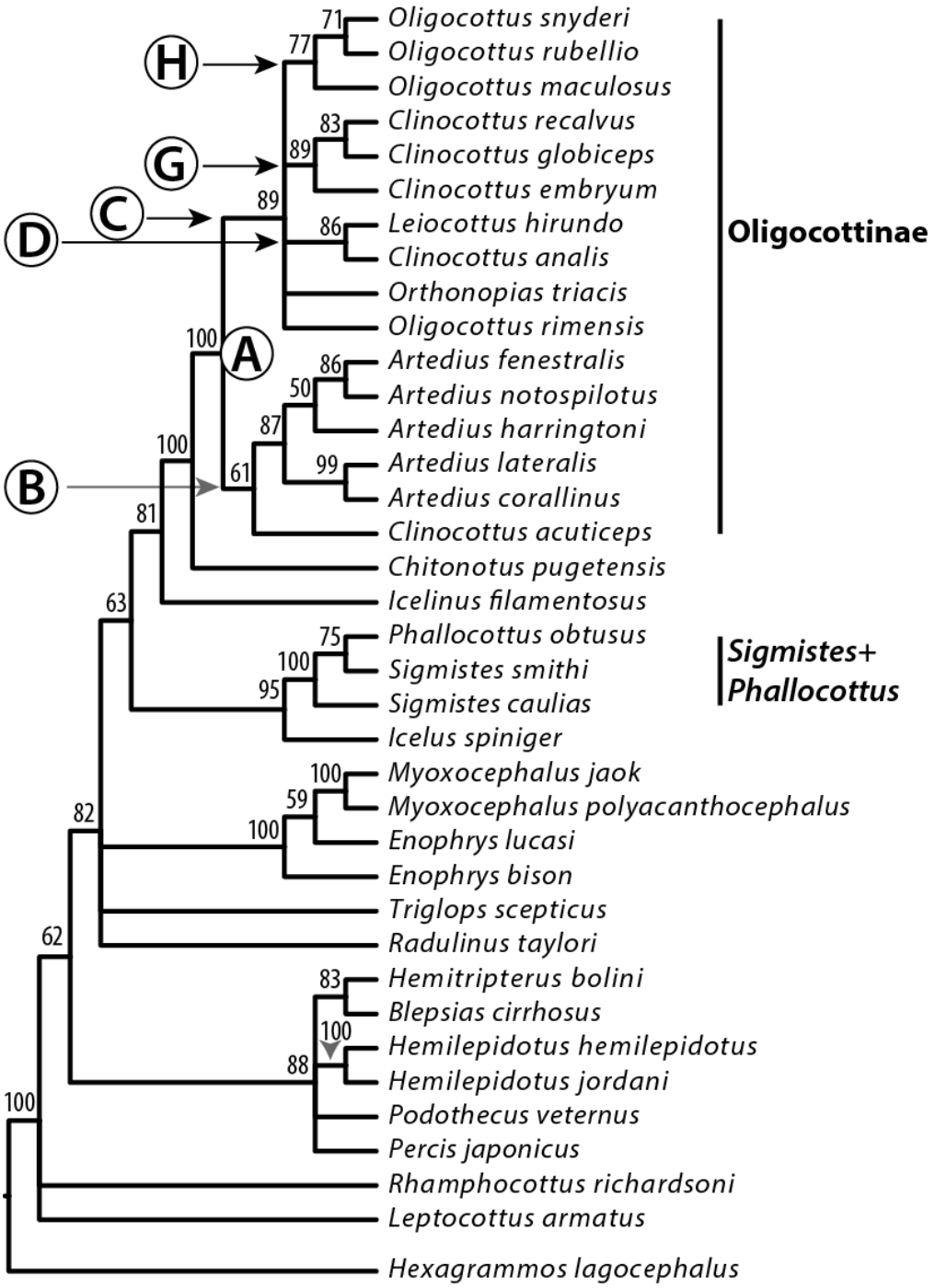

Figure 2.2: Phylogeny of Oligocottinae using parsimony. Phylogeny of oligocottine and outgroup taxa using parsimony analysis of eight concatenated molecular loci, partitioned by locus. This 50% majority-rule consensus tree was generated with 1000 bootstrap

iterations using the same heuristic search strategy as the initial analysis. Support values for each clade are indicated at nodes.

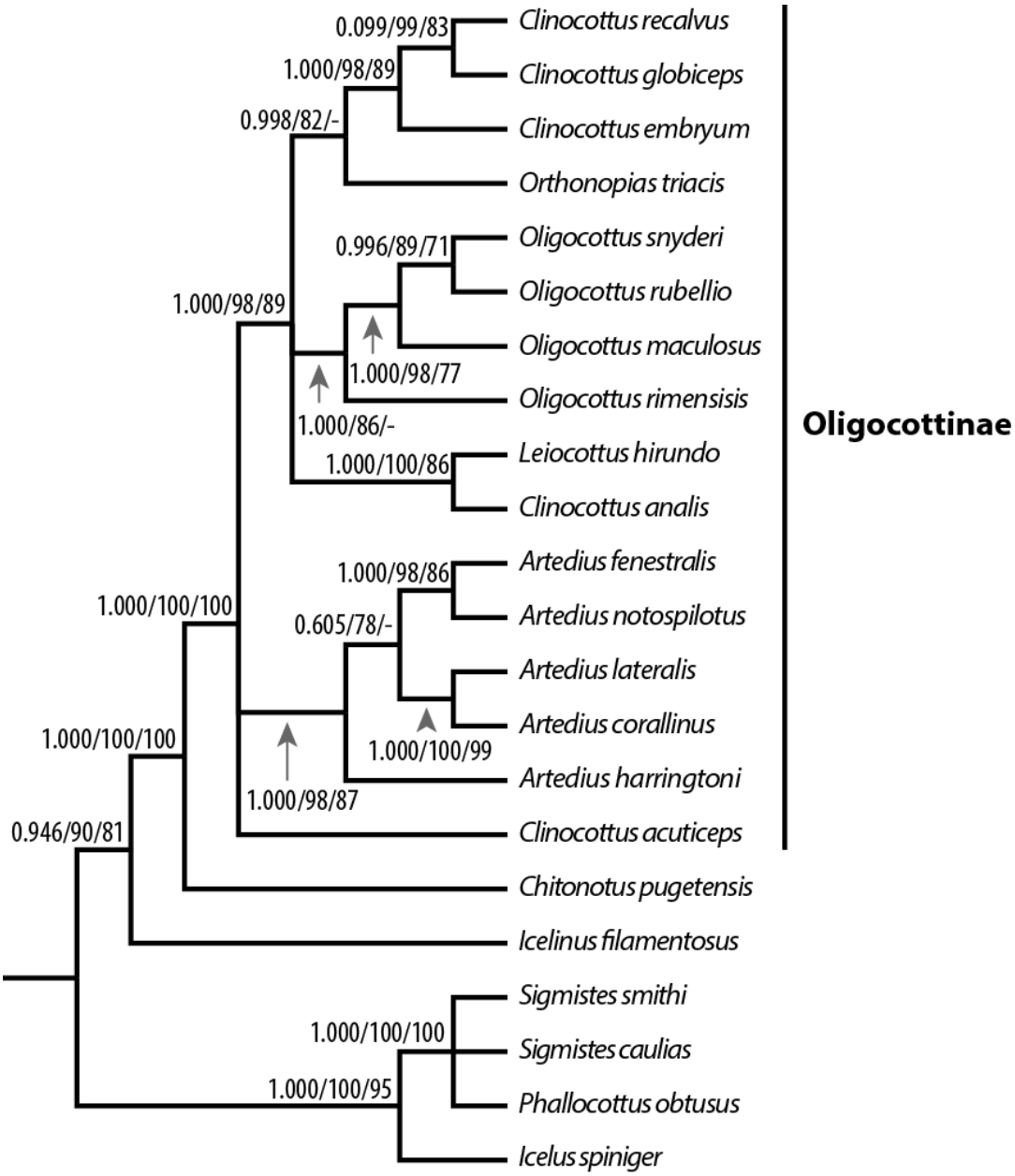

Figure 2.3: Phylogeny of Oligocottinae. Cladogram of oligocottine sculpins showing the most well-supported relationships recovered from phylogenies inferred using maximum likelihood, Bayesian inference, and maximum parsimony. Support values are indicated at each node in the following order: Bayesian posterior probabilities, bootstrap values from maximum likelihood, bootstrap values from parsimony analysis. Dash mark in place of a support value indicates less than 50% support by an analysis for a given node.

2.10 Tables

Table 2.1: Collection data. Collection location, museum identification number, and sample size for all taxa included in this study. Regions are abbreviated as follows: AI = Aleutian Islands, USA; AK = Alaska, USA excluding the Aleutian Islands; BC = British Columbia, Canada; CA = California, USA; OR = Oregon, USA; WA = Washington, USA. Museum identification numbers begin with museum abbreviations as follows: KU = University of Kansas; SIO = Scripps Institution of Oceanography; UAM = University of Alaska Museum; UW = Burke Museum at the University of Washington.

Group	Taxon	n	Catalog Number	Collection Region	Collection Locality
Ingroup					
	Artedius corallinus	1			
			SIO:Fishes:01-124	CA	San Diego
	Artedius fenestralis	3			
			UAM:Fishes:6252	AK	Kodiak Island
			UAM:Fishes:6159	AK	Kasitsna Bay
			UAM:Fishes:6167	AK	Kasitsna Bay
	Artedius harringtoni	6			
			UAM:Fishes:6189	WA	Bremerton
			UAM:Fishes:6186	WA	Bremerton
			UAM:Fishes:6163	AK	Kasitsna Bay
			UAM:Fishes:6155	AK	Kasitsna Bay
			UAM:Fishes:6158	AK	Kasitsna Bay
			UAM:Fishes:4702	CA	Monterey Bay
	Artedius lateralis	5			
			UAM:Fishes:6254	AK	Kodiak Island
			UAM:Fishes:2951	AK	Sitka
			UAM:Fishes:2962	AK	Sitka
			UAM:Fishes:2976	OR	Newport
			UAM:Fishes:2976	OR	Newport

Table 2.1 (…continued): Collection data.

Group	Taxon	n	Catalog Number	Collection Region	Collection Locality
	Artedius notospilotus	1			
			SIO:Fishes:04-2	CA	San Diego
	Clinocottus acuticeps	9			
			UAM:Fishes:6260	AK	Kodiak Island
			UAM:Fishes:6164	AK	Jakolof Bay
			UAM:Fishes:6179	BC	Tofino
			UAM:Fishes:2947	AK	Sitka
			UAM:Fishes:2947	AK	Sitka
			UAM:Fishes:2973	OR	Newport
			UAM:Fishes:2973	OR	Newport
			UAM:Fishes:47693	AI	Attu
			UAM:Fishes:47693	AI	Attu
	Clinocottus analis	5			
			UAM:Fishes:4699	CA	Monterey Bay
			SIO:Fishes:06-42	CA	Cambria
			N/A	CA	Gaviota
			N/A	CA	Gaviota
			N/A	CA	Gaviota
	Clinocottus embryum	8			
			UAM:Fishes:6154	AK	Kasitsna Bay
			UAM:Fishes:6165	AK	Kasitsna Bay
			UAM:Fishes:6154	AK	Kasitsna Bay
			UAM:Fishes:4695	AK	Kodiak Island
			UAM:Fishes:2948	AK	Sitka
			UAM:Fishes:2974	OR	Newport
			UAM:Fishes:47694	AI	Attu
			UAM:Fishes:47694	AI	Attu

Table 2.1 (…continued): Collection data.

Group	Taxon	n	Catalog Number	Collection Region	Collection Locality
	Clinocottus globiceps	6			
			UAM:Fishes:6180	BC	Tofino
			UAM:Fishes:6180	BC	Tofino
			UAM:Fishes:6182	BC	Uculet
			UAM:Fishes:2942	AK	Sitka
			UAM:Fishes:2968	WA	Neah Bay
			UAM:Fishes:2975	OR	Newport
	Clinocottus recalvus	3			
			N/A	CA	Vandenberg Air Force Base
			N/A	CA	Vandenberg Air Force Base
			N/A	CA	Vandenberg Air Force Base
	Oligocottus maculosus	7			
			UAM:Fishes:4698	AK	Prince William Sound
			UAM:Fishes:6259	AK	Kodiak Island
			UAM:Fishes:6188	WA	Bremerton
			UAM:Fishes:6178	BC	Port Hardy
			UAM:Fishes:6166	AK	Kasitsna Bay
			UAM:Fishes:6181	BC	Tofino
			UAM:Fishes:6154	AK	Middleton Island
	Oligocottus rimensis	5			
			UAM:Fishes:2955	AK	Sitka
			UAM:Fishes:2945	AK	Sitka
			UAM:Fishes:2964	AK	Sitka
			UAM:Fishes:2964	AK	Sitka
	Oligocottus rubellio	2			
			N/A	CA	Big Sur
			N/A	CA	Big Sur

Table 2.1 (…continued): Collection data.

Group	Taxon	n	Catalog Number	Collection Region	Collection Locality
	Oligocottus snyderi	9			
			UAM:Fishes:4700	CA	Monterey Bay
			UAM:Fishes:2946	AK	Sitka
			UAM:Fishes:2946	AK	Sitka
			UAM:Fishes:4683	BC	Uculet
			UAM:Fishes:4683	BC	Uculet
			UAM:Fishes:2972	WA	Seiku
			UAM:Fishes:2972	WA	Seiku
			UAM:Fishes:2978	OR	Newport
			UAM:Fishes:2979	OR	Newport
	Orthonopias triacis	4			
			UAM:Fishes:4701	CA	Monterey Bay
			SIO:Fishes:03-166	CA	Carmel
			N/A	CA	Monterey
			N/A	CA	Monterey
	Phallocottus obtusus	2			
			UAM:Fishes:4697	AI	Adak
			UAM:Fishes:4697	AI	Adak
	Sigmistes caulias	6			
			UAM:Fishes:47726	AI	Adak
			UAM:Fishes:47684	AI	Adak
			UAM:Fishes:47715	AI	Tanaga
			UAM:Fishes:47715	AI	Tanaga
			UAM:Fishes:47705	AI	Amchitka
			UAM:Fishes:47706	AI	Amchitka
	Sigmistes smithi	4			
			UAM:Fishes:47712	AI	Ogliuga
			UAM:Fishes:47727	AI	Adak
			UAM:Fishes:47727	AI	Adak
			UAM:Fishes:47727	AI	Adak

Table 2.1 (…continued): Collection data.

Group	Taxon	n	Catalog Number	Collection Region	Collection Locality
Outgroup					
	Blepsias cirrhosus	2			
			UAM:Fishes:2941	AK	Alaska Sea Life Center
			UAM:Fishes:2941	AK	Alaska Sea Life Center
	Chitonotus pugetensis	5			
			UW:Fishes:151078	WA	Puget Sound
			UW:Fishes:151079	WA	Puget Sound
			UW:Fishes:47298	WA	Puget Sound
			UW:Fishes:47675	WA	Myrtle Edwards Park
			UW:Fishes:47676	WA	Myrtle Edwards Park
	Enophrys bison	2			
			UAM:Fishes:6255	AK	Kodiak Island
			UAM:Fishes:6186	WA	Bremerton
	Enophrys lucasi	3			
			UAM:Fishes:6160	AK	Kasitsna Bay
			UAM:Fishes:6160	AK	Kasitsna Bay
			UAM:Fishes:6160	AK	Kasitsna Bay
	Hemilepidotus hemilepidotus	1			
			UAM:Fishes:6177	BC	Smith Sound
	Hemilepidotus jordani	1			
			UAM:Fishes:2938	AK	Alaska Sea Life Center
	Hemitripterus bolini	1			
			UAM:Fishes:2936	AK	Alaska Sea Life Center

Table 2.1 (…continued): Collection data.

Group	Taxon	n	Catalog Number	Collection Region	Collection Locality
	Hexagrammos lagocephalus	2			
			UAM:Fishes:6256	AK	Kodiak Island
			UAM:Fishes:2937	AK	Alaska Sea Life Center
	Icelinus filamentosus	1			
			KU:Fishes:28049	CA	Southern California
	Icelus spiniger	2			
			UAM:Fishes:4703	AK	UNK.
			UAM:Fishes:4703	AK	UNK.
	Leiocottus hirundo	2			
			SIO:Fishes:08-60	CA	San Clemente
			N/A	CA	Los Angeles County
	Leptocottus armatus	2			
			UAM:Fishes:6174	BC	Rivers Inlet
			UAM:Fishes:6174	BC	Rivers Inlet
	Myoxocephalus jaok	1			
			UAM:Fishes:6246	AK	Kodiak Island
	Myoxocephalus polyacanthocephalus	3			
			UAM:Fishes:6257	AK	Kodiak Island
			UAM:Fishes:6257	AK	Kodiak Island
			UAM:Fishes:6168	AK	Kasitsna Bay
	Percis japonicus	1			
			UAM:Fishes:2935	AK	Alaska Sea Life Center
	Podothecus veterus	1			
			UW:Fishes:125588	AK	Bering Sea

Table 2.1 (…continued): Collection data.

Group	Taxon	n	Catalog Number	Collection Region	Collection Locality
	Radulinus taylori	1			
			UAM:Fishes:6191	WA	Bremerton
	Rhamphocottus richardsoni	1			
			UAM:Fishes:2940	AK	Alaska Sea Life Center
	Triglops scepticus	1			
			UAM:Fishes:4704	AK	UNK.

Table 2.2: Genomic loci. Genomic regions, PCR primers, annealing temperatures, and sources of each of the 8 molecular loci used in this study. Sources are as follows: 1 = Betancur et al. 2013, 2 = Campbell et al. 2013, 3 = Chen et al. 2003, 4 = Chen et al. 2008, 5 = Chen et al 2013, 6 = Li et al. 2007, 7 = Li et al. 2010, 8 = This study, 9 = Ward et al. 2005.

Type	Locus	Primer Name	Primer Sequence 5' - 3'	Target Fragment Length (bp)	Anneal Temp.	Source
Mitochondrial						
	COI	FISH_F1	TCAACCAACC ACAAAGACAT TGGCAC	655	52°C	9
		FISH_R1	TAGACTTCTG GGTGGCCAAA GAATCA		52°C	9
Nuclear intron						
	EPIC 1777E4	1777E4F	AGGAGYTGGT GAACCAGAGC AAAGC	300	58°C	7
		1777E4R	AGATCRGCCT GAATSAGCCA GTT		58°C	7
	EPIC 4174E20	4174E20F	CTYTCGCTGG CTTTGTCTCAA ATCA	350	58°C	7
		4174E20R	CTTTTACCATC KCCACTRAAA TCCAC		58°C	7
Nuclear exon						
	EGR1	EGR1 290F	TMTCTTACAC AGGCCGYTTC AC	828	55°C	4
		EGR1 1118R	CTTCTTGTCCT TCTGCCGYAG RT		55°C	5

Table 2.2 (…continued): Genomic loci.

Type	Locus	Primer Name	Primer Sequence 5' - 3'	Target Fragment Length (bp)	Anneal Temp.	Source
	MLL	MLL 1459F	TCCCAGACTC ARGTTTCCAG	711	55°C	2
		MLL 2170R	CTCTGCTGAA KGAGAGTAGT KGG		55°C	2
	ptchd1	ptr458F	AGAATGGATW ACCAACACYT ACG	990	55°C	6
		ptr1248R	TAAGGCACAG GATTGAGATG CT		55°C	6
		ptr463F	GGATAACCAA CACYTACGTC AA	779	62°C	6
		ptr1242R	ACAGGATTGA GATGCTGTCC A		62°C	6
	Rhodopsin	RH 193F	CNTATGAATA YCCTCAGTAC TACC	846	55°C	3
		RH 1039R	TGCTTGTTCAT GCAGATGTAG A		55°C	3
	SVEP	SVEP1 7960F	CCTCCNCAYA TYGAYTTTGG DGAMTA	929	50°C	1
		SVEP1 8889R	TTCAGGWARC CRTGRCTRATR TCCTC		50°C	1
		SVEP 8058F	TCACATTCRTA GCTCACCTTGC TGTTGAAGCC RAACT	652	65°C	8

Table 2.2 (…continued): Genomic loci.

Type	Locus	Primer Name	Primer Sequence 5' - 3'	Target Fragment Length (bp)	Anneal Temp.	Source
		SVEP 8710_R	AGCCCCACCA GGTTRGCGTG YCAGGAG		65°C	8

Table 2.3: Divergence statistics. Trimmed length for each locus is recorded in base pairs (bp).

Locus (trimmed length)	**Group**	**Max distance**	**p-value**	**Mean base frequencies**			
				A	**C**	**G**	**T**
EPIC1777	All	12.01	1.00	24.54	27.68	23.39	24.39
(263 bp)	Ingroup	7.26	1.00	24.42	28.10	23.54	23.94
EPIC4174	All	9.99	1.00	28.60	20.85	19.60	30.96
(283 bp)	Ingroup	5.73	1.00	28.47	20.75	19.52	31.25
COI	All	20.74	1.00	21.68	31.23	19.68	27.41
(651 bp)	Ingroup	20.12	1.00	21.34	31.68	20.02	26.96
EGR1	All	5.24	1.00	20.43	38.70	19.81	21.05
(783 bp)	Ingroup	3.19	1.00	20.38	38.50	19.84	21.28
MLL	All	6.88	1.00	22.95	32.06	22.58	22.41
(693 bp)	Ingroup	4.64	1.00	22.97	32.04	22.55	22.44
ptchd1	All	6.94	1.00	22.27	29.06	24.29	24.38
(678 bp)	Ingroup	4.58	1.00	22.09	29.46	24.43	24.02
Rhodopsin	All	8.41	1.00	15.45	34.18	28.39	21.98
(738 bp)	Ingroup	5.01	1.00	15.16	34.54	28.68	21.62
SVEP	All	11.08	1.00	20.58	25.95	30.92	22.55
(606 bp)	Ingroup	7.59	1.00	20.39	26.27	31.01	22.33
All Loci	All	19.85	0.00	21.32	30.90	23.85	23.93
(4696 bp)	Ingroup	15.25	0.27	21.07	31.27	23.99	23.67

Table 2.4: Best fit partitions and models. Best partitions of molecular dataset and corresponding best fitting models were calculated in PartitionFinder (Lanfear et al. 2012) using the Bayesian information criterion. Protein-coding regions were subdivided by codon position site (cp; i.e., COI was subdivided into COI_cp1, CPIcp2, and COI_cp3). K80 = Kimura 2-parameter (Kimura 1980), F81 = Felsenstein 1981 (Felsentein 1981), HKY = Hasegawa, Kishino, and Yano (Hasegawa et al. 1985), GTR = Generalised Time-Reversible (Tavaré 1986), I = invariable Sites, G = among site rate variation.

Partition	Best model	Loci
1	K80+I+G	EPIC1777E4, EPIC4174E20, MLL_cp1, SVEP_cp1 SVEP_cp2
2	GTR+G	COI_cp1
3	GTR+I+G	COI_cp2, Rhodopsin_cp2, ptchd1_cp2
4	GTR+G	COI_cp3
5	F81+I	EGR1_cp1, EGR1_cp2, MLL_cp2, ptchd1_cp1
6	GTR+G	EGR1_cp3, ptchd1_cp3
7	K80+I	MLL_cp3
8	K80+I	Rhodopsin_cp1
9	HKY+G	Rhodopsin_cp3
10	HKY+G	SVEP_cp3

Table 2.5: Proposed revisions to taxonomy and classification.

Historical taxonomy and classification	Proposed revision
Leiocottus hirundo	*Clinocottus hirundo*
Subgenus *Clinocottus* (*sensu* Bolin 1944)	Subgenus *Clinocottus*
C. analis	*C. analis*
	C. hirundo
Oligocottini (*sensu* Taranets 1941)	Oligocottini
Clinocottus	*Clinocottus*
Oligocottus	*Oligocottus*
Sigmistes	*Orthonopias*
Oligocottinae (*sensu* Taranets 1941)	Oligocottinae
Artedius	*Artedius*
Clinocottus	*Clinocottus*
Oligocottus	*Oligocottus*
Orthonopias	*Orthonopias*
Sigmistes	

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Chapter 3: Distribution of reproductive traits within Oligocottinae[2]

3.1 Abstract

I constructed an ultrametric phylogenetic tree using the dataset generated for the phylogenetic study described in Chapter 2. Using this phylogenetic framework, I conducted an analysis of the evolution of reproductive behaviors and associated morphological characters in members of Oligocottinae. I reviewed the literature on the reproductive biology of oligocottine sculpins to identify pertinent traits. From this review I generated a matrix of character states that were mapped onto a phylogeny of oligocottine sculpins. Ancestral state reconstruction was used to explore their evolution. The results show that copulation and the presence of an enlarged male genital papilla are likely the ancestral states of Oligocottinae and that these characters were secondarily lost in the lineage composed of *Artedius corallinus*, *A. fenestralis*, *A. lateralis*, and *A. notospilotus*. The results also show that parental care in the group is split between the *Artedius* lineage, where males guard egg clutches, and the rest of the group, where egg guarding does not occur. It is possible that the differing ecology of these two groups has affected the evolution of reproduction and parental care in the subfamily, where subtidal lineages (i.e., *Artedius*) engage in parental care but have transitioned away from copulation, while the intertidal lineages maintained copulation but hide their eggs rather than guard them.

3.2 Introduction

The sculpins (Scorpaeniformes; Cottoidea) are a group of approximately 380 species of fishes distributed primarily across the northern hemisphere (Nelson 2006). This superfamily is notable for its radiation into many diverse habitats (i.e., rivers, lakes, cave systems, intertidal, deep-sea, etc. see Mecklenburg et al. 2002, Espinasa and Jeffery 2003) and the wide variety of reproductive strategies found within the group (Yokoyama and Goto, 2005, Abe and Munehara 2009, Munoz 2010). Especially notable among the diversity of sculpin reproductive traits is a mode of fertilization called internal gamete

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association with delayed fertilization (IGA), which is found only in cottoids (Abe and Munehara 2009). IGA is associated with a range of morphological and behavioral specializations that vary by species and include: the presence of an intromittent organ, nest-building, parental care, and clasping structures used to facilitate copulation. Some species display all of these specializations, while others display few or other, even more bizarre specializations, such as the eversible genital tract found in female *Hemitripterus villosus* (Pallas 1814) (Krejsa 1964, Morris 1956, Munehara et al. 1989, Munehara et al. 1991, Munehara 1996, Petersen et al. 2005).

Previous studies (i.e., Abe and Munehara 2009, Munoz 2010) have attempted to explore the evolution and diversity of copulation and associated behaviors (e.g., parental care) among sculpins by mapping the distribution of reproductive traits onto a phylogeny of Cottoidea. These studies showed that the distribution of copulation and parental care is widespread and disparate across the superfamily. However, the phylogenetic hypothesis used in these studies (i.e., Yabe 1985) has been called into question by recent molecular-based studies (e.g., Knope 2013) as well as by morphology-based analyses (Jackson 2003). Indeed, Jackson (2003) re-analyzed the data presented in Yabe (1985) and concluded that the phylogeny presented in Yabe (1985) was markedly different (in terms of interfamilial relationships and the resolution of relationships among the genera examined) than a strict consensus of the most parsimonious trees generated from that dataset. Jackson (2003) noted that the tree topology was unstable, likely as a result of the high number of taxa (n=59) and a relatively low number of parsimony-informative characters (n=36). A well-supported phylogenetic hypothesis should be considered prerequisite to exploring the distribution of traits within a given group. Given that such a phylogeny is not available for Cottoidea, any patterns that may be evident in the distribution and evolution of reproductive traits among sculpins should be considered unknown or speculative and would require an intensive effort to produce a robust hypothesis of phylogenetic relationships among all cottoids. Pending the availability of a comprehensive phylogenetic hypothesis, focused study of reproduction in a well-

circumscribed subset of cottoids may yield insights on the factors associated with different reproductive characteristics.

The subfamily Oligocottinae (*sensu* Chapter 2) is a monophyletic group that includes both copulating and non-copulating species, making it an ideal group in which to explore the evolution of reproductive biology in sculpins. The objectives of this study were to map the known distribution of reproductive traits within the subfamily Oligocottinae and use ancestral state reconstruction to infer the evolution of reproductive traits within the group.

3.3 Methods

3.3.1 Character states and data matrix

Ten morphological and behavioral characters were examined among the 16 species of oligocottine sculpins, as well as the following outgroups: *Chitonotus pugetensis* (Steindachner 1876), *Icelinus filamentosus* (Gilbert 1890), *Icelus spiniger* Gilbert 1896, *Sigmistes caulias* Rutter 1898 (in Jordan and Evermann 1898), *S. smithi* Schultz 1938, and *Phallocottus obtusus* Schultz 1938. Character state codings for each species were determined from existing descriptions of morphology and/or behavior in the literature. Where possible, character conditions were also verified by examination of museum specimens (see Table 3.1). The characters and character states that comprise the data matrix are listed and described below:

3.3.2 Characters and character states

1. **Enlarged male genital papilla (0 = Absent, 1= Present)**. This character was coded verbatim from the literature and verified by examination of museum specimens (see Table 3.1 for sample sizes per species). For the purposes of verification, the size of the male genital papilla was compared to that of the females. Sex was determined by examination of secondary sexual characteristics or of gonads.
2. **Position of vent (0 = abutting insertion of anal fin, 1 = anterior of anal fin insertion)**. The vent was defined as the smallest possible area that encompassed

the genital papilla and the anal papilla. This character was coded using descriptions in the literature and verified by examination of museum specimens (see Table 3.1 for sample sizes per species). For verification, the position was considered "anterior" if the distance between the vent and the anal fin insertion was greater than or equal to the diameter of the vent. For species where males posses an enlarged genital papilla, only females were used to determine the position of the vent.

3. **Spermatozoon morphology (0 = oval, 1 = intermediate, 2 = slender)**. Character states were adapted and coded from the descriptions in Hann (1930). Hann (1930) determined sperm morphology from examination of fixed tissue (testes), which had been dehydrated, embedded in paraffin, cut to a thickness of 8 to 10 microns, and stained in iron-hematoxylin and eosin. See Hann (1930) for full description of methods.
4. **Morphology of testes (0 = anterior duct, 1 = anterior duct vesicle, 2 = non-duct, 3 = non-duct vesicle, 4 = posterior duct)**. Character states were adapted and coded from the descriptions in Koya et al. (2011). To determine morphology of the testes, Koya et al. (2011) first fixed gonads in Bouin's solution (or fixed in formalin and later transferred to Bouin's solution) then examined them by eye and photographed them. Testes were later dehydrated, embedded in paraffin, sectioned to a thickness of 6 microns, and stained with Delafield's hematoxylin and eosin. The internal structure of the testes was thus determined using light microscopy. See Koya et al. (2011) for full description of methods.
5. **Parental care (0 = absent, 1 = present)**. Nest building and/or egg guarding by one or both parents were considered forms of parental care. The presence of parental care, where known, was determined from a review of behavior descriptions found in multiple independent studies that each used snorkel, SCUBA, and/or aquarium observations. See Table 3.1 for specific references.
6. **Copulation (0 = absent, 1 = present)**. Copulation was defined as the transfer of sperm from a male into the ovarian cavity of a female. The presence of

copulation, where known, was determined from a review of behavior descriptions found in multiple independent studies that each used snorkel, SCUBA, and/or aquarium observations. See Table 3.1 for specific references.

3.3.3 Phylogenetic framework

Specimens representing all species of the genera: *Oligocottus* Girard 1856, *Clinocottus* Gill 1861, *Sigmistes* Rutter 1898 (in Jordan and Evermann 1898), *Artedius* Girard 1856, *Phallocottus* Schultz 1938, *Leiocottus* Girard 1856, and *Orthonopias* Starks and Mann 1911 as well as the outgroups: *Chitonotus pugetensis*, *Icelinus filamentosus*, *Icelus spiniger*, *Sigmistes caulias*, *S. smithi*, and *Phallocottus obtusus* were assembled from field and museum collections.

Sculpins were collected from nearshore and intertidal habitats from 34 localities across Alaska, British Columbia, Washington, and Oregon (Table 3.2). Collections were made from intertidal habitats using dip nets at low tide and from sub-tidal habitats using SCUBA equipment. Voucher specimen and tissue samples are archived in the fish collections at University of Alaska Museum and the University of Washington. In addition to directed collections, specimens and/or tissue samples were provided by the Alaska Sea Life Center, Mayumi Arimitsu (United States Geological Survey), Milton Love (University of California, Santa Barbara), Marina Ramon (University of Southern California), Scripps Institution of Oceanography, University of Washington Fish Collection, and the University of Kansas. In total, 96 individuals representing 22 species were included in this study (Table 3.2).

3.3.4 DNA sequence determinations

Total genomic DNA was extracted from fin and muscle tissue with reagents and protocols from the DNEasy Blood and Tissue Kit (Qiagen Corp.). DNA fragments from eight molecular loci: one mitochondrial protein-coding locus (Cytochrome *c* oxidase, COI), two nuclear introns [exon-primed intron crossing (EPIC) locus 1777E10 and EPIC locus 4174E20] and five protein-coding nuclear loci [early growth response protein 1 (EGR1); mixed-lineage leukemia (MLL); patched domain-containing protein 1 (ptchd1);

Rhodopsin; and Sushi, von Willebrand factor type A, and pentraxin domain-containing 1 (SVEP) were amplified by targeted polymerase chain reactions (PCR) according to the protocol outlined in Chapter 2.

Amplicons were purified and sequenced in both directions by Sanger sequencing at the University of Washington High-Throughput Genomics Unit. Sequences were trimmed, visually checked for quality, and assembled into forward-reverse contiguous sequences using CodonCode Aligner Software (CodonCode Corp.) Multiple sequence alignments (MSAs) for each locus were generated in ClustalW (Larkin et al. 2007). Alignments were trimmed and reading frame established using Se-Al (Rambaut 2002). MSAs for all loci were concatenated using Mesquite (Maddison and Maddison 2011). Models of molecular evolution were tested for each locus independently using MrModeltest (Nylander 2004) using the Akaike information criterion (AIC; Akaike 1973, Posada and Buckley 2004).

In order to capture intra-specific sequence variability, the full dataset contained multiple individuals for many species. For the purposes of character mapping and ancestral state reconstruction, however, a chimera sequence was generated for each species using the most common allele/haplotype among individuals of that species at each locus in order to reduce the representation of each species to a single tip. In order to test for any effects of using the chimera sequences, phylogenetic trees were produced for both the full dataset and the chimera-sequence dataset using identical phylogenetic inferences methods.

To generate an ultrametric tree on which to map characters and infer ancestral states, Bayesian analysis of each concatenated dataset was conducted in BEAST v. 1.7.5 (Drummond et al. 2012). For each locus, the model of molecular evolution yielding the lowest AIC value (as calculated in MrModeltest) was applied. The rate of molecular evolution was modeled as an uncorrelated lognormal relaxed clock (see Drummond et al. 2006) and was unlinked across all loci. All tree models shared a birth-death speciation tree prior. Four independent analyses were run for 50 million generations each and were sampled every 10,000 generations. MCMC logs were visualized using Tracer v. 1.5

(Rambaut and Drummond 2007) to determine convergence and an appropriate number of generations to discard as burn-in. Burn-in was removed and trees were combined using LogCombiner v. 1.7.5 (http://beast.bio.ed.ac.uk/LogCombiner). A maximum clade credibility (MCC) tree was produced from the combined trees using TreeAnnotator v. 1.7.5 (http://beast.bio.ed.ac.uk/TreeAnnotator). This process was completed for both the full dataset and the chimera-sequence dataset and the topology of the two trees were compared with one another.

3.3.4 Character mapping and ancestral state reconstruction

Character states were mapped onto the chimera-sequence MCC tree using Mesquite 2.75 (Maddison and Maddison 2011). Ancestral states were reconstructed using maximum likelihood with the Markov k-state 1 parameter model of evolution (see Lewis 2001). In this model, all potential changes in state are equally probable (that is, the probability of state 1 changing to state 2 is equal to the probability of state 1 changing to state 3).

3.4 Results

3.4.1 Sequences

To account for length variation following alignment, the MSAs for each locus were trimmed to the following lengths: COI = 651 bp, EPIC locus 1777E10 = 263 bp, EPIC locus 4174E20 = 283 bp, EGR1 = 783 bp, MLL = 693 bp, ptchd1 = 678 bp, Rhodopsin = 738 bp, and SVEP =606 bp, for a total of 4696 aligned nucleotide sites, of which 1037 were variable and, of those, 368 were parsimony-informative.

For each locus, the following models of molecular evolution have the lowest AIC values and therefore represent the best fit: EPIC locus 1777E4 and SVEP best fit the General Time Reversible (GTR) model (Tavaré 1986) with a four category gamma-distribution; COI, ptchd1, and Rhodopsin best fit the GTR model with a four category gamma-distribution and invariable sites; EPIC locus 4174E20 and MLL best fit the Hasegawa-Kishino-Yano (HKY) model (Hasegawa et al. 1985) with a four category gamma-distribution; and EGR1 best fit the HKY model with gamma-distribution and

invariable sites. These models were used in the phylogenetic analyses of the concatenated dataset.

3.4.2 Phylogenetic framework

The phylogenies produced from analysis of the full dataset and the chimera-sequence dataset had very similar topologies to one another, differing only in the placement of *Clinocottus acuticeps* (Gilbert 1896). In the analysis of the full dataset, *C. acuticeps* was placed as sister to the Oligocottini (*sensu* Chapter 2). In the analysis of the chimera sequences, *C. acuticeps* was placed as sister to the genus *Artedius* (Fig. 3.1). In both analyses, the support for the placement of *C. acuticeps* was low (<0.900 Bayesian posterior probability (Bpp)). The topology of the chimera-sequence phylogeny differed from previous analyses of the full dataset using maximum likelihood and Bayesian inference (Chapter 2: Fig. 2.1) only in the placement of the outgroup taxon *Phallocottus obtusus*, the placement of which had low support values in all analyses (Chapter 2: Figures 2.1 and 2.2).

3.4.3 Character mapping and ancestral state reconstruction

The distribution of characters states for all taxa examined in this study was summarized into a data matrix (Table 3.3). There was considerable variation in the size and morphology of the genital papillae that were described as “enlarged” in the literature (Fig. 3.2). However, the amount of variation was extensive and further classification of these papillae into discrete categories would necessarily be arbitrary. This character contained the only instance where physical observation of specimens contradicted previous morphological descriptions: male *Orthonopias triacis* Starks and Mann 1911 are described as having a genital papilla that is “not enlarged” (Bolin 1944). Yet, there is clearly an external genital papilla in some males (Fig. 3.3)

For many behavioral traits (e.g., copulation- Character 6) or internal structures (e.g. morphology of the testes- Character 4), descriptions were only available for a few species (Table 3.3), making reconstruction of the ancestral states subject to change with the addition of new observations.

1. **Enlarged male genital papilla:** The presence of this trait was highly likely in the ancestor of oligocottine sculpins (0.996 proportional likelihood (pl)) and appears to have been secondarily lost in *Artedius fenestralis* Jordan and Gilbert 1883, *A. notospilotus* Girard 1856, *A. lateralis* Girard 1854, and *A. corallinus* (Hubbs 1926), which together will be referred to as clade J (Fig. 3.4).
2. **Position of vent relative to anal fin insertion:** The distribution of this trait appears to split among oligocottine genera. Members of the genera *Oligocottus* and *Artedius* have a vent that abuts the insertion of the anal fin, while members of *Clinocottus*, *Leiocottus*, and *Orthonopias* have an anteriorly advanced vent (Fig. 3.5). The ancestor of Oligocottinae was more likely to have an advanced vent (0.762 pl) than an abutting vent (0.238 pl).
3. **Spermatozoon morphology:** Within Oligocottinae, oval-shaped spermatozoa appear to be entirely restricted to clade J in the genus *Artedius* (Fig. 3.6) Slender-shaped spermatozoa have been described in all members of *Clinocottus* and *Orthonopias*, and all but one species of *Oligocottus*. *O. maculosus* Girard 1856 and *A. harringtoni* are the only oligocottine sculpins to possess an intermediate spermatozoon morphology. The slender spermatozoon morphology is the most likely (0.881 pl) state for the ancestor of oligocottine sculpins.
4. **Morphology of testes:** Data were very limited for this character (see Table 3.3). *Artedius fenestralis* and *A. lateralis* posses anterior duct type testes. *A. harringtoni* and *Oligocottus maculosus* possess anterior duct vesicle type testes. *Clinocottus recalvus* possesses non-duct type testes.
5. **Parental care:** This trait was strongly split between the tribe Oligocottini and *Artedius*, with parental care being found only in members of the latter (Fig. 3.7). There is no clear signal as to the ancestral state of this character for oligocottine sculpins, but it does appear highly likely that parental care was present in the ancestor of *Artedius*, and not present in the ancestor of the Oligocottini.
6. **Copulation:** Copulation is likely (0.869 pl) an ancestral state for Oligocottinae (Fig. 3.8). Copulation is present in all oligocottine sculpins whose reproductive

biology has been described, with the exception of *A. lateralis* and *A. fenestralis*. The absence of this trait from clade J appears to have been a secondary loss (Fig. 3.8).

3.5 Discussion

The results of these analyses show that the ancestral states for Oligocottinae are likely: an enlarged male genital papilla (Fig. 3.4), an anteriorly advanced vent (Fig. 3.5), a slender-type spermatozoon morphology (Fig. 3.6), and copulation (Fig. 3.8). There is also strong support for an enlarged male genital papilla as the ancestral condition of the oligocottine sculpins (Fig. 3.4). The results also show that the ancestral states for clade J (which is nested within Oligocottinae) are likely a non-enlarged male genital papilla, an unadvanced vent, oval-type spermatozoon morphology, and absence of copulation.

Previous studies of the evolution of reproductive modes in sculpins (i.e., Abe and Munehara 2009, Munoz 2010) have postulated that the evolution of copulation in sculpins had been sporadic and polyphyletic as evidenced by the scattered appearance of copulatory behavior on the phylogenetic framework proposed by Yabe (1985). The evidence presented here shows that, at least in Oligocottinae, copulation and associated morphological characters is very likely to be ancestral and that lineages that lack the trait have lost it secondarily.

Within Oligocottinae, many character states have become polarized between the *Artedius* and oligocottinin lineages. *Artedius* is the only oligocottine group where parental care has been observed (Fig. 3.7) and clade J, within *Artedius*, has the only occurrences of oligocottine species that do not copulate or possess an enlarged male genital papilla (Figures 3.4, 3.8). Clade J also contains the only oligocottine occurrences of posterior duct type testes and oval shaped spermatozoa (Table 3.3). Other sculpins that possess these kinds of testis (i.e., *Hemilepidotus* spp. and *Gymnocanthus* spp.) and sperm (i.e. *Cottus* spp.) also do not copulate (Hann 1930, Morris 1952, Savage 1963, Goto 1982, Koya et al. 2011). However, *A. fenestralis* and *A. lateralis* have demonstrated a kind of facultative insemination where spermatozoa appear to inconsistently enter the ovarian cavity of females and associate with eggs as a result of external spawning activity

(see Petersen et al. 2005). In fact, the spermatozoa in *A. lateralis* and *A. fenestralis* have been shown to be active in both full strength seawater and seawater that has been diluted to one-third strength seawater (which approximates the osmolality of the ovarian fluid of these species; see Petersen et al. 2005). For comparison, the spermatozoa of *A. harringtoni* and *O. maculosus* (two copulating species) have been observed to only be active in the diluted seawater (Petersen et al 2005). The results of this study suggest that this likely represents the evolution of a more seawater-adapted sperm type in clade J, associated with the loss of copulation.

In contrast, the tribe Oligocottini seems to represent the opposite end of the spectrum, where enlarged genital papillae, slender-type spermatozoon morphology, and copulation are retained (Figures 3.4, 3.6, 3.8). Corroborating this finding is the observation that the morphology of testis and sperm found in Oligocottini have, to date, only been found in other species of copulating sculpins (Hann 1930, Koya et al. 2011). Further separating Oligocottini from *Artedius* is the complete absence of parental care among oligocottinin sculpins (Fig. 3.7). Given the available data, it was not possible to confidently determine the most likely ancestral state of parental care for Oligocottinae. However, the presence of parental care is common and widespread throughout Cottoidei (Munoz 2010), and it is my opinion that the ancestors of oligocottine sculpins exhibited parental care as well. However, determining with certainty whether parental care was lost in Oligocottini or gained in *Artedius* will depend on the results of future studies into the reproductive behavior of oligocottine sculpins and their close allies.

The only trait that does not cleanly split between the Oligocottini lineage and *Artedius* is the position of the vent relative to the anal fin insertion. Rather, the distribution of this trait was split between *Oligocottus* + *Artedius* and the rest of Oligcottinae (Fig. 3.5). This distribution closely matches the distribution of greatly enlarged male genital papillae. With the exception of *Orthonopias triacis*, all species of sculpins examined in this study (including the outgroup taxa) that have an anteriorly advanced vent also possessed a greatly enlarged male genital papilla (Figures 3.2, 3.4, 3.5). By contrast, those species with a vent that abutted the insertion of the anal fin either

lacked an enlarged papilla, or the papilla was small and/or threadlike. This correlation could represent a close relationship of the two features. This relationship is seemingly likely as, in live individuals, the papillae typically face posteriorly (pers. obs.), and could inhibit the function of the anal fin if the two structures were abutting one another.

Complex traits such as IGA result from of the evolution of a number of related morphological and, frequently, behavioral traits (e.g. Stearns 1977, Johnston and Page 1992, Quinn et al. 2001, Goddard and Hayes 2009). Within Oligocottinae, copulation and associated physical traits appear to have been greatly reduced in the *Artedius* line and maintained in the Oligocottini group. Conversely, parental care in the form of egg-guarding is well documented in *Artedius*, but has never been observed in any member of Oligocottini. The polarization of these traits among oligocottine sculpins may be related to ecological factors. For instance, *Artedius* is primarily a sub-tidal group, while Oligocottini is made up of primarily intertidal species (pers. obs., see descriptions in Bolin 1944, Mecklenburg et al. 2002). Egg-hiding (i.e., selective spawning in an out-of-the-way place) is common among the non-guarding, copulating sculpins (e.g.. *Hemitripterus villosus*, Munehara 1992; *Pseudoblennius* spp., Shinomiya 1985). This behavior has been described in some oligocottine sculpins as well: female *O. maculosus* have been observed to spawn their eggs in rocky crevices (Atkinson 1939), and female *C. acuticeps* are known to spawn in the high intertidal (3.0 -3.7m above mean sea level) under *Fucus* sp. (Marliave 1981). With regard to *C. acuticeps*, the cover of algae has been shown to inhibit desiccation of the developing eggs (Marliave 1981). Depositing eggs in the high intertidal may reduce the risk of egg-predation by subtidal, invertebrate predators (such as pandalid shrimp- see Petersen 2005 for an account of shrimp predation on *A. fenestralis* egg clutches following the removal of the guarding male). High-intertidal spawning is seen in other fishes, such as California grunion, *Leuresthes tenuis* (Walker 1949), and members of the killifish genus *Fundulus* (Taylor 1990, 1999). At least in the case of *C. acuticeps*, spawning in the high intertidal may alleviate the need to guard eggs altogether. Another factor that may have influenced the lack of parental care in the intertidal oligocottinin sculpins is the risk of predation on adults from terrestrial

predators. Pacific staghorn sculpin, *Leptocottus armatus* Girard 1854, are commonly found in intertidal habitats (Mecklenburg et al. 2002, pers. obs.) and have been shown to make up a large proportion (~37%) of the diet of coastal great blue herons (*Ardea herodias* Linnaeus 1758), a large wading bird that hunts fishes by stalking them in shallow water (Krebs 1974). Parental care can increase the risk of predation on the parent, especially when the size of the parent is relatively small (Magnhagen 1992). It is possible then that wading birds or other terrestrial-based predators are simply too great a threat to sculpins that inhabit shallow, intertidal depths to make egg-guarding practical for these fishes.

The unique evolutionary forces present in intertidal vs. subtidal habitats have undoubtedly influenced the evolution of the oligocottine sculpins found in each and may have contributed to the polarized distribution of reproductive traits found within the subfamily.

3.6 Conclusions

Copulation and IGA are likely the ancestral states of Oligocottinae. This conclusion is supported by the ancestral state reconstruction of enlarged male genital papillae (Fig. 3.4), spermatozoon morphology (Fig. 3.6), and copulation (Fig. 3.8). Ancestral state reconstruction also shows quite clearly that these characters were secondarily lost in clade J, the lineage composed of *Artedius corallinus*, *A. fenestralis*, *A. lateralis*, and *A. notospilotus*. The morphology of the testis of oligocottine sculpins (Table 3.3, see Koya et al. 2011) matches the distribution seen in the other characters examined in this study. It is possible that as some of the oligocottine lineages transitioned from subtidal to intertidal habitats, they began to encounter a different suite of selective pressures than their subtidal relatives. It may be this rift that led the subtidal lineages to maintain parental care but transition away from copulation, while the intertidal lineages maintained copulation but quickly transitioned away from egg guarding to egg hiding.

It is conceivable that copulation and IGA evolved very early in sculpins and the modern, apparently disparate distribution of these traits is due to secondary losses. The ability to trace the origin of these traits has been hindered by the lack of a well-supported,

highly resolved phylogeny of sculpins and the many gaps in our current understanding of sculpin reproductive biology. Given the diversity of reproductive strategies found among sculpins, the potential to explore complex patterns of evolution is great, yet tangible, in this group. Within Cottoidea, there is at least one group that has evolved internal fertilization (i.e., *Comephorus* spp.), one species where the female has evolved an intromittent organ (i.e., *Hemilepidotus villosus*), several species where males have evolved an intromittent organ (e.g., *Clinocottus* spp., *Enophrys* spp., *Icelinus* spp., *Sigmistes* spp.), and several species where males have evolved structures used for grasping females during copulation (e.g., *Oligocottus* spp., *Radulinopsis* spp.). There are also examples of egg hiding (e.g.,*Pseudoblennius* spp.) , paternal parental care (e.g., *Cottus* spp.), and maternal parental care (e.g., *Radulinopsis taranetzi*, Yabe and Maruyama 2001). The forces that have driven the evolution of parental care and other reproductive traits in this group are certainly not unique. Understanding the ways in which these fishes have changed as a result of those forces would undoubtedly shed more light on how these complex traits have evolved in other groups as well.

3.7 Acknowledgements

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and Use Committee. Fishes were collected under: Alaska Department of Fish and Game Fish Resource Permit #CF-11-094, #CF-12-037, #CF-12-042, #CF-13-028, and #CF-13-082; Department of Fisheries and Oceans Canada Scientific Permit #XR_305_2011, #XR_308_2011, and #XR_269_2012; Washington Department of Fish and Wildlife Scientific Collection Permit #11-365; and Oregon Department of Fish and Wildlife Scientific Taking Permit #17221.

3.7 Figures

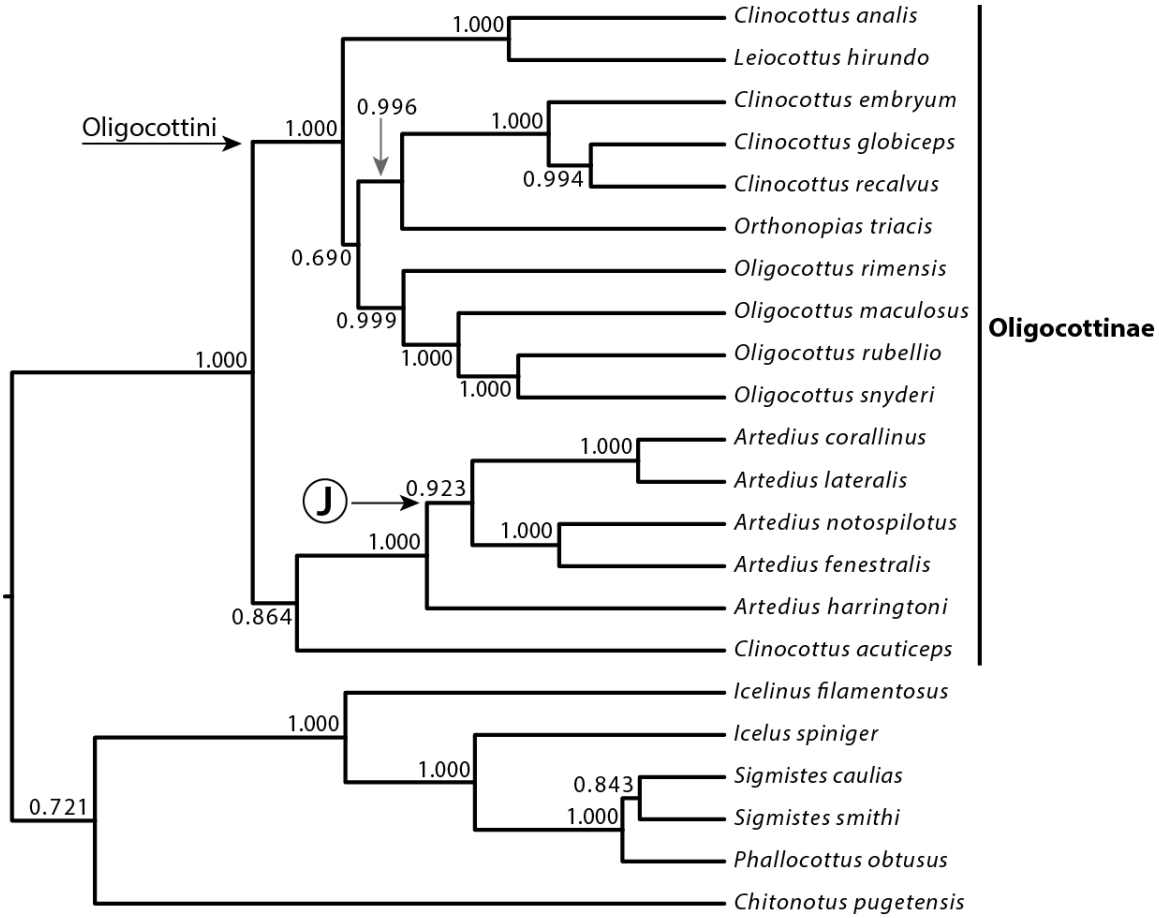

Figure 9: Maximum clade credibility phylogeny of Oligocottinae. Phylogeny was constructed using Bayesian inference of the partitioned analysis of eight molecular loci (EPIC locus 1777E4, EPIC locus 4174E20, COI, EGR1, MLL, ptchd1, Rhodopsin, and SVEP) in BEAST. Bayesian posterior probability scores were generated with four independent runs of 50 million generations each and are indicated at each node. The tribe Oligocottini and the subset of the genus *Artedius* referred to as clade J in the text are each indicated with an arrow pointing to the base of the respective clades.

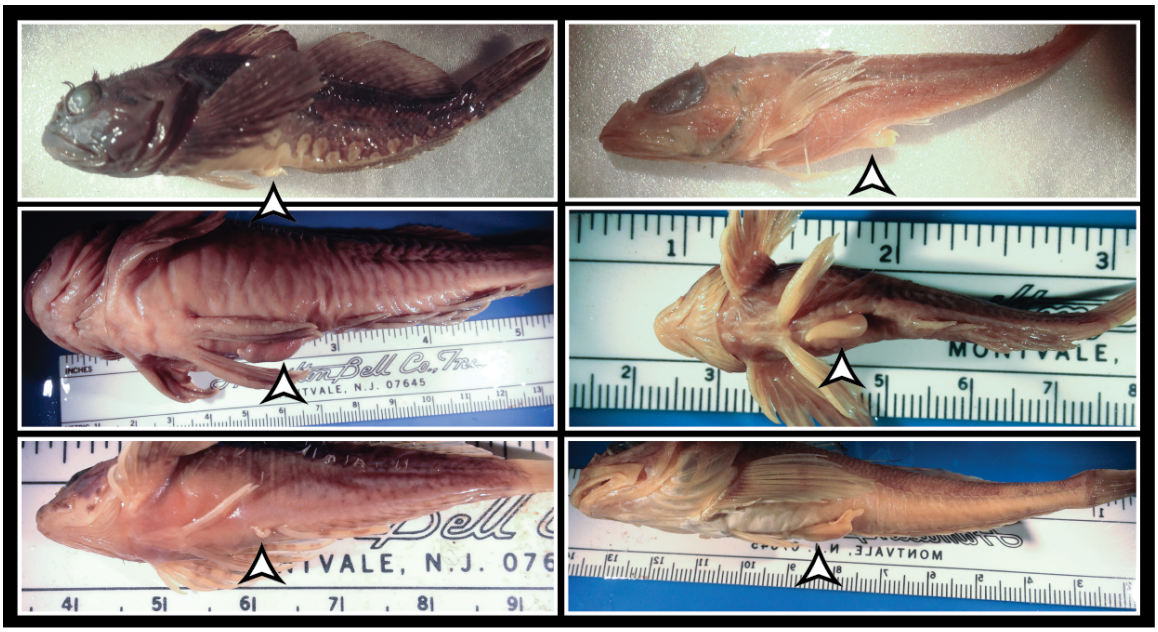

Figure 10: Morphological variation in enlarged male genital papillae. Arrows indicate location of genital papilla. Species and museum lot numbers are as follows, starting from the upper right and moving clockwise: *Artedius harringtoni*, UAM_6163; *Icelus spiniger*, UAM_1537; *Clinocottus embryum*, OSU_7071; *Chitonotus pugetensis*, OSU_7016; *Oligocottus rubellio*, OSU_8133; *Clinocottus globiceps*, OSU_3204. Lot numbers begin with a three letter code which indicates the location of the specimen: OSU = Oregon State University, UAM = University of Alaska Museum.

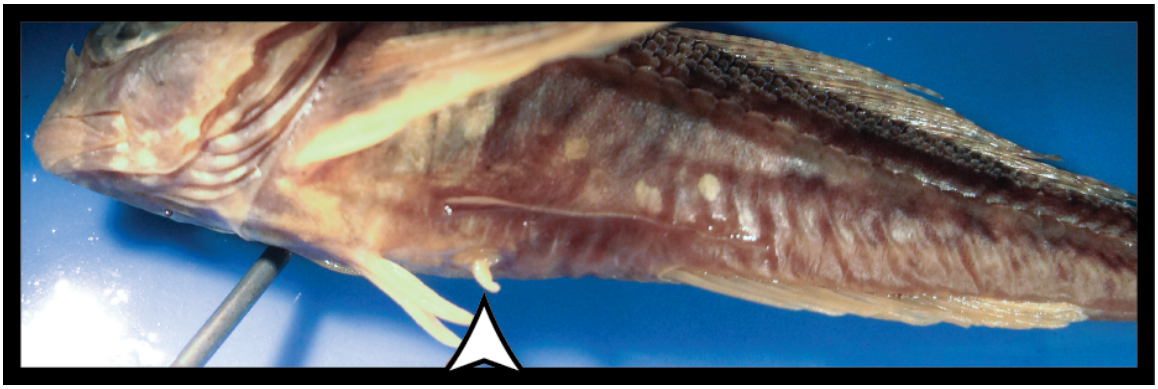

Figure 3.3: Genital papilla of male *Orthonopias triacis*. The location of the papilla is indicated by an arrow. Specimen is located at the Oregon State University Ichthyological Collection, catalog number 8137.

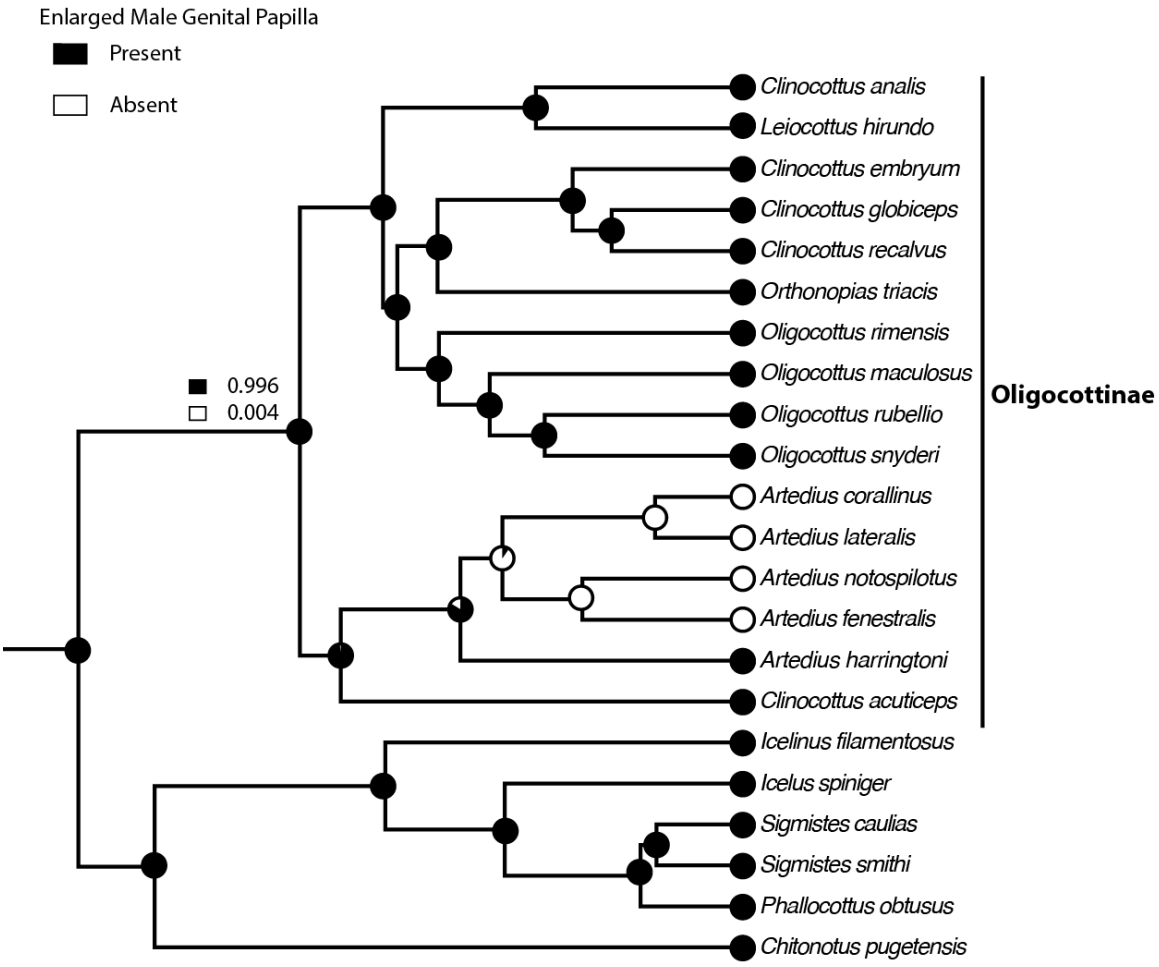

Figure 3.4: Ancestral state reconstruction of enlarged male genital papilla (Character 1). The proportional likelihood of the presence vs. absence of an enlarged male genital papilla for the ancestor of a given clade is depicted with a pie chart at each respective node. The proportion of the area of the pie filled with the color black corresponds to the proportional likelihood of presence of the trait; the white area corresponds to the proportional likelihood of absence of the trait. Additionally, the proportional likelihood of the presence and absence of the trait for the ancestor of Oligocottinae is specified at the base of the clade. Phylogenetic framework was inferred by a partitioned analysis of eight molecular loci (EPIC locus 1777E4, EPIC locus 4174E20, COI, EGR1, MLL, ptchd1, Rhodopsin, and SVEP) in BEAST. The tribe Oligocottini and the subset of the genus *Artedius* referred to as clade J in the text are each indicated with an arrow pointing to the base of the respective clades.

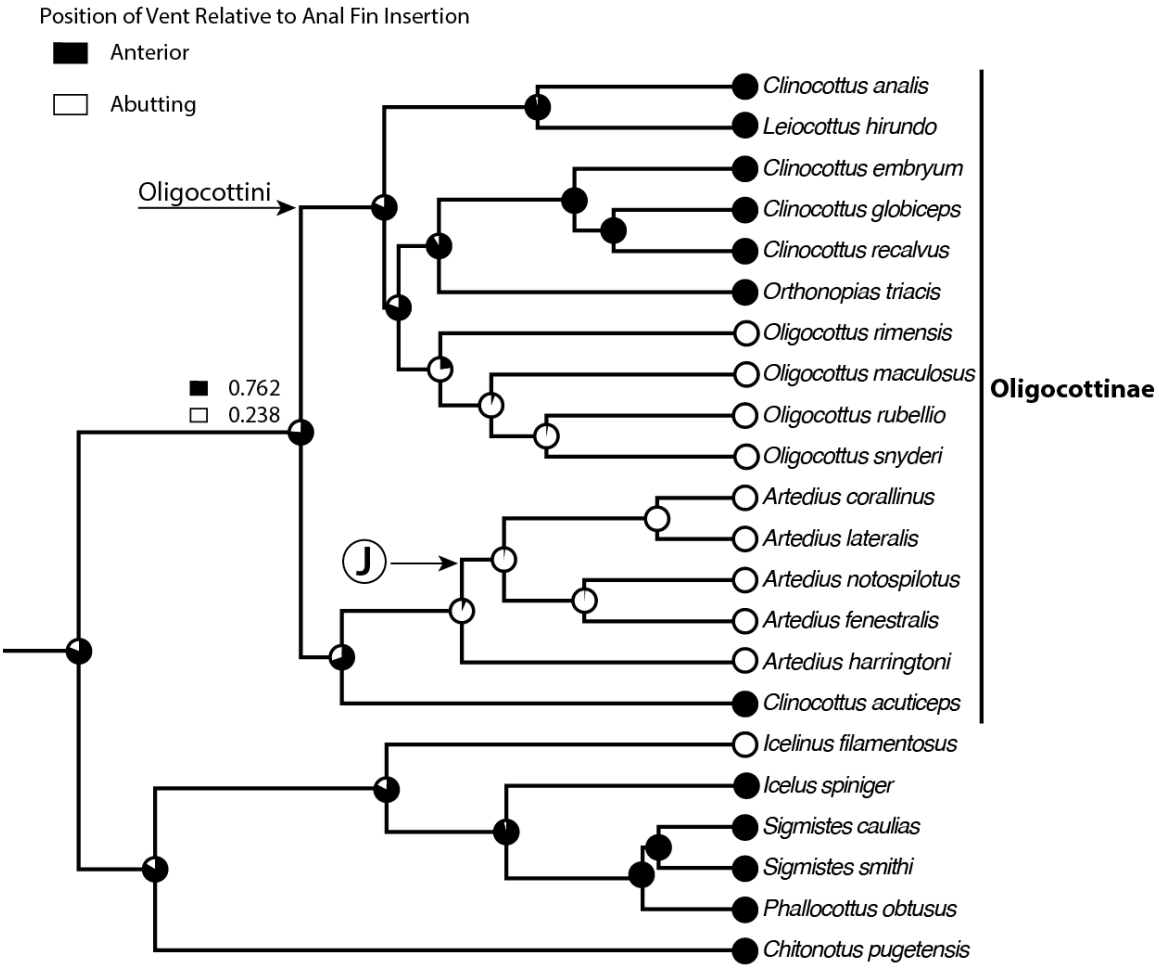

Figure 3.5: Ancestral state reconstruction of vent position (Character 2). The proportional likelihood of the relative position of the vent for the ancestor of a given clade is depicted with a pie chart at each respective node. The proportion of the area of the pie filled with the color black corresponds to the proportional likelihood of an anteriorly placed vent; the white area corresponds to the proportional likelihood of a vent that abuts the insertion of the anal fin. Additionally, the proportional likelihoods of the relative position of the vent for the ancestor of Oligocottinae is specified at the base of the clade. Phylogenetic framework was inferred by a partitioned analysis of eight molecular loci (EPIC locus 1777E4, EPIC locus 4174E20, COI, EGR1, MLL, ptchd1, Rhodopsin, and SVEP) in BEAST. The tribe Oligocottini and the subset of the genus *Artedius* referred to as clade J in the text are each indicated with an arrow pointing to the base of the respective clades.

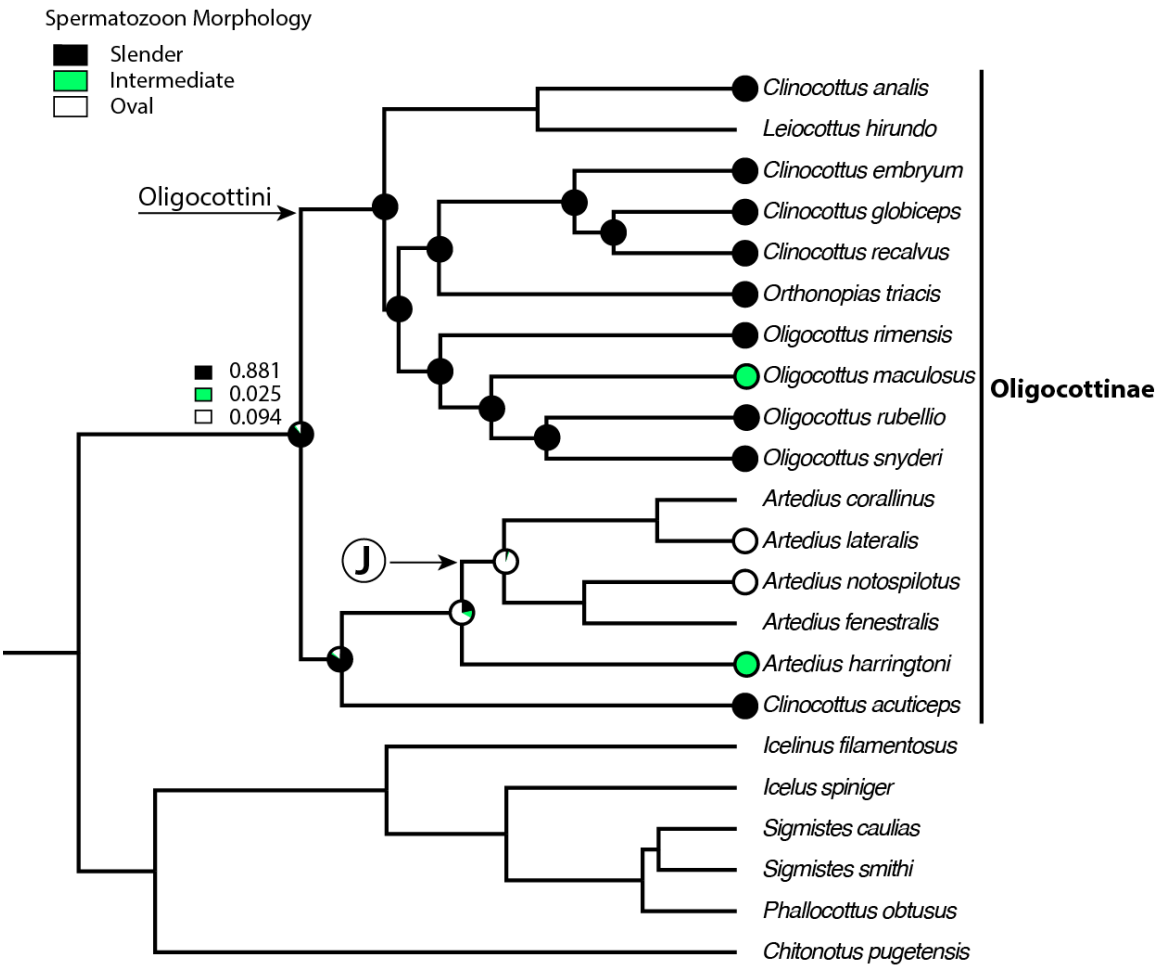

Figure 3.6: Ancestral state reconstruction of spermatozoon morphology (Character 3). The proportional likelihood of the type of spermatozoon present in the ancestor of a given clade is depicted with a pie chart at each respective node. Nodes without pie charts indicate missing/unknown data. The proportion of the area of the pie filled with the color black corresponds to the proportional likelihood of the slender-type spermatozoon; the area of the pie filled with the color green corresponds to the proportional likelihood of the intermediate spermatozoon; and the white area corresponds to the proportional likelihood of the oval-type spermatozoon. Additionally, the proportional likelihoods of the spermatozoon type for the ancestor of Oligocottinae is specified at the base of the clade. Phylogenetic framework was inferred by a partitioned analysis of eight molecular loci (EPIC locus 1777E4, EPIC locus 4174E20, COI, EGR1, MLL, ptchd1, Rhodopsin, and

SVEP) in BEAST. The tribe Oligocottini and the subset of the genus *Artedius* referred to as clade J in the text are each indicated with an arrow pointing to the base of the respective clades.

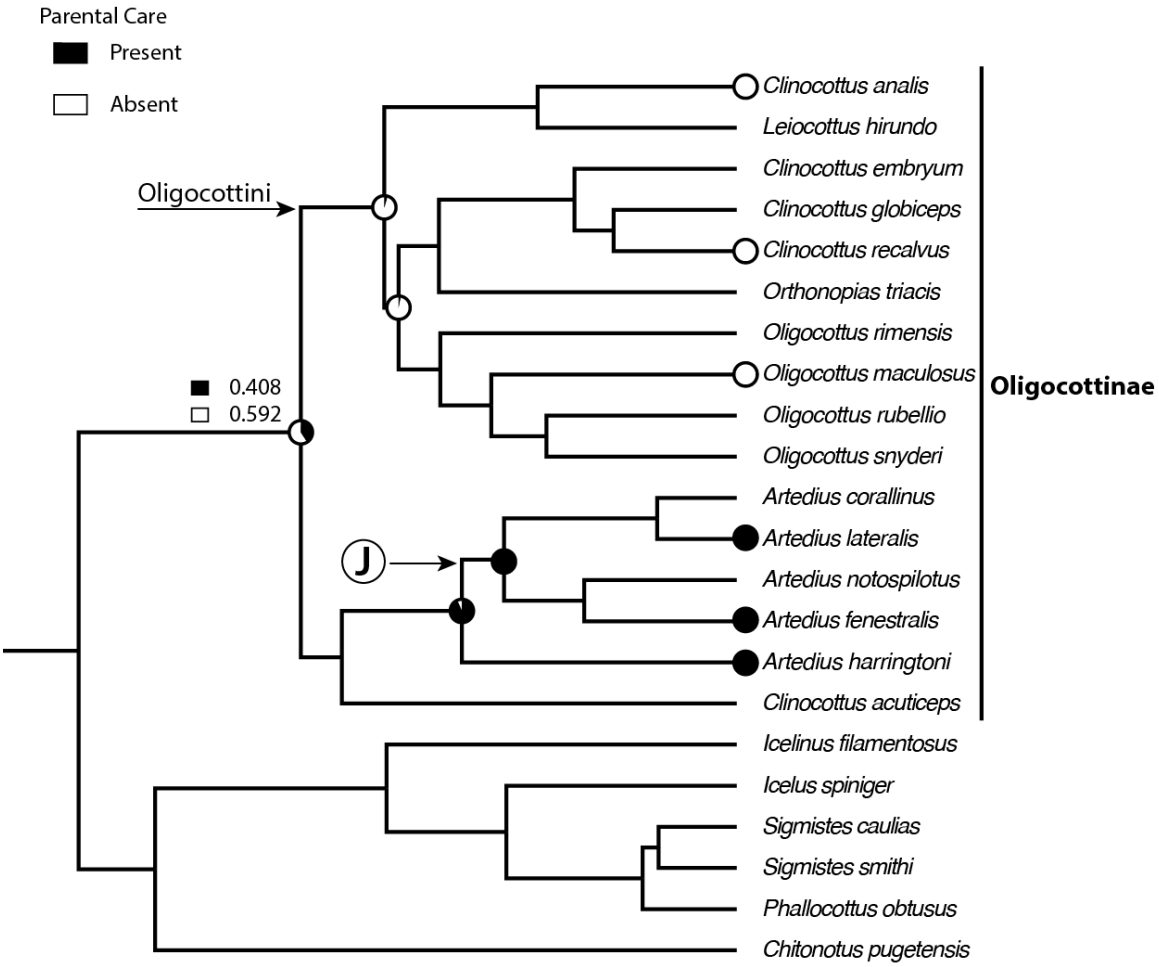

Figure 3.7: Ancestral state reconstruction of parental care (Character 5). The proportional likelihood of the presence vs. absence of parental care for the ancestor of a given clade is depicted with a pie chart at each respective node. Nodes without pie charts indicate missing/unknown data. The proportion of the area of the pie filled with the color black corresponds to the proportional likelihood of the presence of parental care; the white area corresponds to the proportional likelihood of the absence of parental care. Additionally, the proportional likelihood of the presence and absence of the trait for the ancestor of Oligocottinae is specified at the base of the clade. Phylogenetic framework was inferred by a partitioned analysis of eight molecular loci (EPIC locus 1777E4, EPIC locus 4174E20, COI, EGR1, MLL, ptchd1, Rhodopsin, and SVEP) in BEAST. The tribe Oligocottini and the subset of the genus *Artedius* referred to as clade J in the text are each indicated with an arrow pointing to the base of the respective clades.

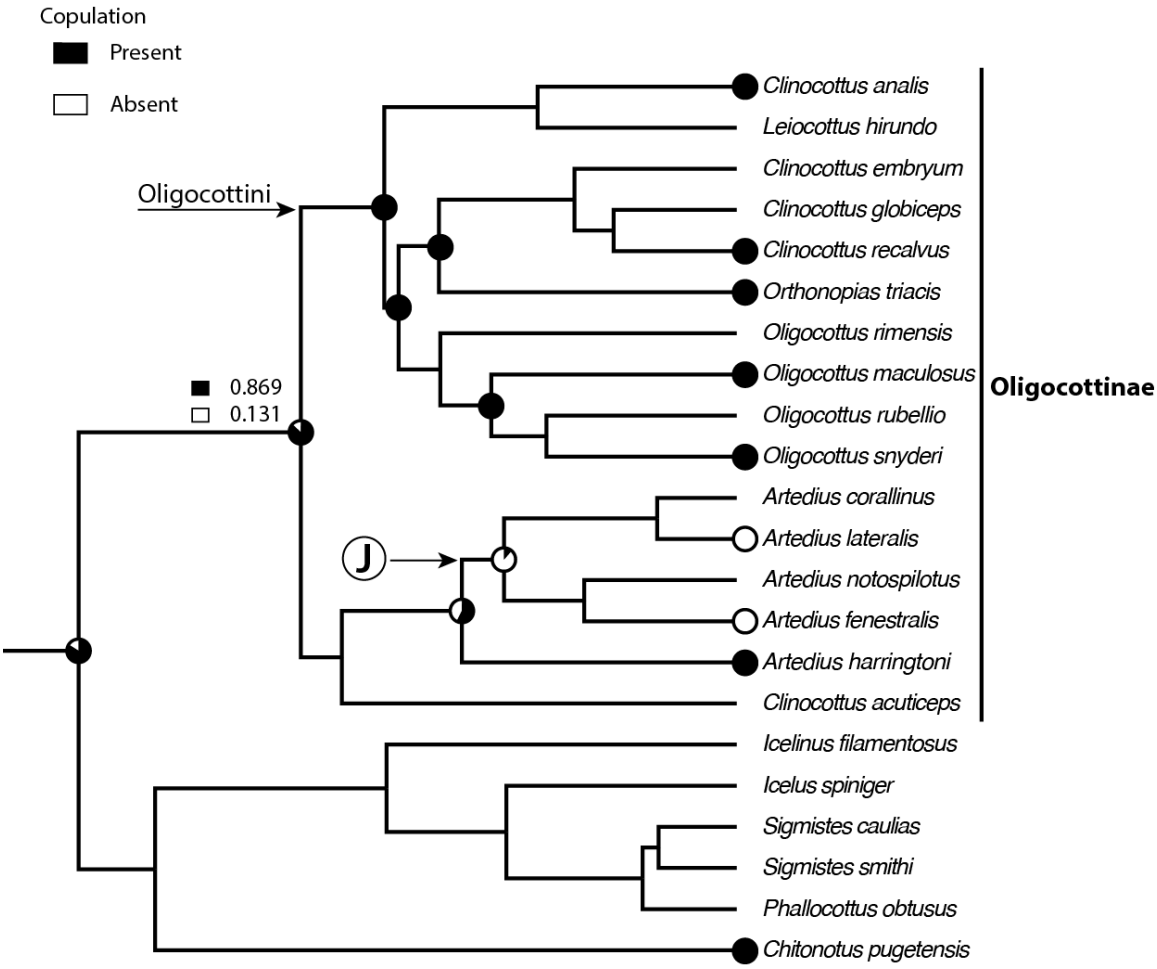

Figure 3.8: Ancestral state reconstruction of copulation (Character 6). The proportional likelihood of the presence vs. absence of copulation for the ancestor of a given clade is depicted with a pie chart at each respective node. Nodes without pie charts indicate missing/unknown data. The proportion of the area of the pie filled with the color black corresponds to the proportional likelihood of the presence of copulation; the white area corresponds to the proportional likelihood of the absence of copulation. Additionally, the proportional likelihood of the presence and absence of the trait for the ancestor of Oligocottinae is specified at the base of the clade. Phylogenetic framework was inferred by a partitioned analysis of eight molecular loci (EPIC locus 1777E4, EPIC locus 4174E20, COI, EGR1, MLL, ptchd1, Rhodopsin, and SVEP) in BEAST. The tribe Oligocottini and the subset of the genus *Artedius* referred to as clade J in the text are each indicated with an arrow pointing to the base of the respective clades.

3.8 Tables

Table 3.1: Materials examined. Museum lot numbers begin with museum abbreviations as follows: OSU = Oregon State University, SIO = Scripps Institution of Oceanography, UAM = University of Alaska Museum. In many cases, multiple individuals were examined from a single lot.

Taxon	Number examined	Museum catalog numbers
Artedius corallinus	13	OSU:Fishes:8140
Artedius fenestralis	24	UAM:Fishes:0713, UAM:Fishes:0714, UAM:Fishes:0715, UAM:Fishes:2950, UAM:Fishes:2957, UAM:Fishes:2961, UAM:Fishes:5196, UAM:Fishes:5197, UAM:Fishes:5198, UAM:Fishes:5199, UAM:Fishes:6159, UAM:Fishes:6167, UAM:Fishes:6252
Artedius harringtoni	7	UAM:Fishes:4702, UAM:Fishes:6155, UAM:Fishes:6158, UAM:Fishes:6163, UAM:Fishes:6189, UAM:Fishes:6184, UAM:Fishes:6253
Artedius lateralis	28	UAM:Fishes:1745, UAM:Fishes:2345, UAM:Fishes:2943, UAM:Fishes:2951, UAM:Fishes:2962, UAM:Fishes:2970, UAM:Fishes:2976, UAM:Fishes:3506, UAM:Fishes:4688, UAM:Fishes:6190, UAM:Fishes:6185, UAM:Fishes:6254
Artedius notospilotus	1	OSU:Fishes:3508
Chitonotus pugetensis	29	OSU:Fishes:001354, OSU:Fishes:004803, OSU:Fishes:005269, OSU:Fishes:006352, OSU:Fishes:006542, OSU:Fishes:007016, OSU:Fishes:011335, OSU:Fishes:011501, OSU:Fishes:011749, OSU:Fishes:011756, OSU:Fishes:014869, OSU:Fishes:014872
Clinocottus acuticeps	50	UAM:Fishes:2947, UAM:Fishes:2965, UAM:Fishes:2966, UAM:Fishes:2973, UAM:Fishes:4679, UAM:Fishes:4684, UAM:Fishes:4689, UAM:Fishes:4693, UAM:Fishes:4694, UAM:Fishes:4699, UAM:Fishes:6156, UAM:Fishes:6164, UAM:Fishes:6179, UAM:Fishes:6248, UAM:Fishes:47677, UAM:Fishes:47678, UAM:Fishes:47680, UAM:Fishes:47682, UAM:Fishes:47689, UAM:Fishes:47693, UAM:Fishes:47696, UAM:Fishes:47713, UAM:Fishes:47718
Clinocottus analis	33	OSU:Fishes:00914, OSU:Fishes:02716, OSU:Fishes:06710, OSU:Fishes:08136

Table 3.1 (…continued): Materials examined.

Taxon	Number Examined	Museum Catalog Numbers
Clinocottus embryum	50	UAM:Fishes:2948, UAM:Fishes:2954, UAM:Fishes:2967, UAM:Fishes:2974, UAM:Fishes:4680, UAM:Fishes:4690, UAM:Fishes:4695, UAM:Fishes:47681, UAM:Fishes:47683, UAM:Fishes:47686, UAM:Fishes:47690, UAM:Fishes:47694, UAM:Fishes:47695, UAM:Fishes:47697, UAM:Fishes:47701, UAM:Fishes:47704, UAM:Fishes:47707, UAM:Fishes:47714, UAM:Fishes:47719, UAM:Fishes:47722
Clinocottus globiceps	32	UAM:Fishes:2942, UAM:Fishes:2968, UAM:Fishes:2975, UAM:Fishes:4681, UAM:Fishes:4685, UAM:Fishes:4691, UAM:Fishes:6180, UAM:Fishes:6182
Clinocottus recaluvs	4	UAM:Fishes:0663, UAM:Fishes:2002, UAM:Fishes:2305, UAM:Fishes:2342
Icelinus filamentosus	10	OSU:Fishes:000918, OSU:Fishes:001232, OSU:Fishes:003911, OSU:Fishes:004509, OSU:Fishes:011541
Icelus spiniger	50	OSU:Fishes:008757, OSU:Fishes:008759, UAM:Fishes:0389, UAM:Fishes:0404, UAM:Fishes:0409, UAM:Fishes:0416, UAM:Fishes:0450, UAM:Fishes:0451, UAM:Fishes:0452, UAM:Fishes:0456, UAM:Fishes:0484, UAM:Fishes:0671, UAM:Fishes:0672, UAM:Fishes:1537, UAM:Fishes:1839, UAM:Fishes:2784, UAM:Fishes:2785, UAM:Fishes:3051, UAM:Fishes:3395, UAM:Fishes:3410, UAM:Fishes:4703, UAM:Fishes:5209, UAM:Fishes:5210, UAM:Fishes:5211, UAM:Fishes:5212, UAM:Fishes:5213
Leiocottus hirundo	2	OSU:Fishes:8132
Oligocottus maculosus	50	CAS:Fishes:22542, CAS:Fishes:212692, UAM:Fishes:2944, UAM:Fishes:2952, UAM:Fishes:2959, UAM:Fishes:2963, UAM:Fishes:2969, UAM:Fishes:2971, UAM:Fishes:2977, UAM:Fishes:4682, UAM:Fishes:4686, UAM:Fishes:4692, UAM:Fishes:4696, UAM:Fishes:4698, UAM:Fishes:6176, UAM:Fishes:6178, UAM:Fishes:6181, UAM:Fishes:6183, UAM:Fishes:6188, UAM:Fishes:6250, UAM:Fishes:6259, UAM:Fishes:6157, UAM:Fishes:6162, UAM:Fishes:6166, UAM:Fishes:6169, UAM:Fishes:6170, UAM:Fishes:6171
Oligocottus rimensis	23	OSU:Fishes:08138, UAM:Fishes:2955, UAM:Fishes:2964

Table 3.1 (…continued): Materials examined.

Taxon	Number Examined	Museum Catalog Numbers
Oligocottus rubellio	50	CAS:Fishes:19502, CAS:Fishes:28461, CAS:Fishes:25190, CAS:Fishes:19799, CAS:Fishes:212624
Oligocottus snyderi	50	CAS:Fishes:19635, CAS:Fishes:28412, CAS:Fishes:33156, CAS:Fishes:84164, CAS:Fishes:212653, SU:Fishes:48073, UAM:Fishes:2946, UAM:Fishes:2946, UAM:Fishes:2953, UAM:Fishes:2972, UAM:Fishes:2978, UAM:Fishes:4683, UAM:Fishes:4687, UAM:Fishes:4700
Orthonopias triacis	8	OSU:Fishes:08137, UAM:Fishes:4701
Phallocottus obtusus	2	UAM:Fishes:4697
Sigmistes caluias	50	UAM:Fishes:47684, UAM:Fishes:47688, UAM:Fishes:47692, UAM:Fishes:47705, UAM:Fishes:47706, UAM:Fishes:47711, UAM:Fishes:47715, UAM:Fishes:47721, UAM:Fishes:47726
Sigmistes smithi	4	UAM:Fishes:47712, UAM:Fishes:47716, UAM:Fishes:47727

Table 3.2: Collection data. Collection location, museum identification number, and sample size for all taxa included in this study. Regions are abbreviated as follows: AI = Aleutian Islands, USA; AK = Alaska, USA excluding the Aleutian Islands; BC = British Columbia, Canada; CA = California, USA; OR = Oregon, USA; WA = Washington, USA. Museum identification numbers begin with museum abbreviations as follows: KU = University of Kansas; SIO = Scripps Institution of Oceanography; UAM = University of Alaska Museum; UW = Burke Museum at the University of Washington.

Group	Taxon	n	Catalog number	Collection region	Collection locality
Ingroup					
	Artedius corallinus	1			
			SIO:Fishes:01-124	CA	San Diego
	Artedius fenestralis	3			
			UAM:Fishes:6252	AK	Kodiak Island
			UAM:Fishes:6159	AK	Kasitsna Bay
			UAM:Fishes:6167	AK	Kasitsna Bay
	Artedius harringtoni	6			
			UAM:Fishes:6189	WA	Bremerton
			UAM:Fishes:6186	WA	Bremerton
			UAM:Fishes:6163	AK	Kasitsna Bay
			UAM:Fishes:6155	AK	Kasitsna Bay
			UAM:Fishes:6158	AK	Kasitsna Bay
			UAM:Fishes:4702	CA	Monterey Bay
	Artedius lateralis	5			
			UAM:Fishes:6254	AK	Kodiak Island
			UAM:Fishes:2951	AK	Sitka
			UAM:Fishes:2962	AK	Sitka
			UAM:Fishes:2976	OR	Newport
			UAM:Fishes:2976	OR	Newport

Table 3.2 (…continued): Collection data.

Group	Taxon	n	Catalog number	Collection region	Collection locality
	Artedius notospilotus	1			
			SIO:Fishes:04-2	CA	San Diego
	Clinocottus acuticeps	9			
			UAM:Fishes:6260	AK	Kodiak Island
			UAM:Fishes:6164	AK	Jakolof Bay
			UAM:Fishes:6179	BC	Tofino
			UAM:Fishes:2947	AK	Sitka
			UAM:Fishes:2947	AK	Sitka
			UAM:Fishes:2973	OR	Newport
			UAM:Fishes:2973	OR	Newport
			UAM:Fishes:47693	AI	Attu
			UAM:Fishes:47693	AI	Attu
	Clinocottus analis	5			
			UAM:Fishes:4699	CA	Monterey Bay
			SIO:Fishes:06-42	CA	Cambria
			N/A	CA	Gaviota
			N/A	CA	Gaviota
			N/A	CA	Gaviota
	Clinocottus embryum	8			
			UAM:Fishes:6154	AK	Kasitsna Bay
			UAM:Fishes:6165	AK	Kasitsna Bay
			UAM:Fishes:6154	AK	Kasitsna Bay
			UAM:Fishes:4695	AK	Kodiak Island
			UAM:Fishes:2948	AK	Sitka
			UAM:Fishes:2974	OR	Newport
			UAM:Fishes:47694	AI	Attu
			UAM:Fishes:47694	AI	Attu

Table 3.2 (…continued): Collection data.

Group	Taxon	n	Catalog number	Collection region	Collection locality
	Clinocottus globiceps	6			
			UAM:Fishes:6180	BC	Tofino
			UAM:Fishes:6180	BC	Tofino
			UAM:Fishes:6182	BC	Uculet
			UAM:Fishes:2942	AK	Sitka
			UAM:Fishes:2968	WA	Neah Bay
			UAM:Fishes:2975	OR	Newport
	Clinocottus recalvus	3			
			N/A	CA	Vandenberg Air Force Base
			N/A	CA	Vandenberg Air Force Base
			N/A	CA	Vandenberg Air Force Base
	Leiocottus hirundo	2			
			SIO:Fishes:08-60	CA	San Clemente
			N/A	CA	Los Angeles County
	Oligocottus maculosus	7			
			UAM:Fishes:4698	AK	Prince William Sound
			UAM:Fishes:6259	AK	Kodiak Island
			UAM:Fishes:6188	WA	Bremerton
			UAM:Fishes:6178	BC	Port Hardy
			UAM:Fishes:6166	AK	Kasitsna Bay
			UAM:Fishes:6181	BC	Tofino
			UAM:Fishes:6154	AK	Middleton Island
	Oligocottus rimensis	5			
			UAM:Fishes:2955	AK	Sitka
			UAM:Fishes:2945	AK	Sitka
			UAM:Fishes:2964	AK	Sitka
			UAM:Fishes:2964	AK	Sitka

Table 3.2 (…continued): Collection data.

Group	Taxon	n	Catalog number	Collection region	Collection locality
	Oligocottus rubellio	2			
			N/A	CA	Big Sur
			N/A	CA	Big Sur
	Oligocottus snyderi	9			
			UAM:Fishes:4700	CA	Monterey Bay
			UAM:Fishes:2946	AK	Sitka
			UAM:Fishes:2946	AK	Sitka
			UAM:Fishes:4683	BC	Uculet
			UAM:Fishes:4683	BC	Uculet
			UAM:Fishes:2972	WA	Seiku
			UAM:Fishes:2972	WA	Seiku
			UAM:Fishes:2978	OR	Newport
			UAM:Fishes:2979	OR	Newport
	Orthonopias triacis	4			
			UAM:Fishes:4701	CA	Monterey Bay
			SIO:Fishes:03-166	CA	Carmel
			N/A	CA	Monterey
			N/A	CA	Monterey
Outgroup					
	Chitonotus pugetensis	5			
			UW:Fishes:151078	WA	Puget Sound
			UW:Fishes:151079	WA	Puget Sound
			UW:Fishes:47298	WA	Puget Sound
			UW:Fishes:47675	WA	Myrtle Edwards Park
			UW:Fishes:47676	WA	Myrtle Edwards Park
	Icelinus filamentosus	1			
			KU:Fishes:28049	CA	Southern California

Table 3.2 (…continued): Collection data.

Group	Taxon	n	Catalog number	Collection region	Collection locality
	Icelus spiniger	2			
			UAM:Fishes:4703	AK	UNK.
			UAM:Fishes:4703	AK	UNK.
	Phallocottus obtusus	2			
			UAM:Fishes:4697	AI	Adak
			UAM:Fishes:4697	AI	Adak
	Sigmistes caulias	6			
			UAM:Fishes:47726	AI	Adak
			UAM:Fishes:47684	AI	Adak
			UAM:Fishes:47715	AI	Tanaga
			UAM:Fishes:47715	AI	Tanaga
			UAM:Fishes:47705	AI	Amchitka
			UAM:Fishes:47706	AI	Amchitka
	Sigmistes smithi	4			
			UAM:Fishes:47712	AI	Ogliuga
			UAM:Fishes:47727	AI	Adak
			UAM:Fishes:47727	AI	Adak
			UAM:Fishes:47727	AI	Adak

Table 3.3: Data matrix. The following characters were examined in Oligocottine sculpins and several outgroup taxa: 1) enlarged male genital papilla, 2) position of vent relative to anal fin insertion, 3) spermatozoon morphology, 4) morphology of testes, 5) parental care, 6) copulation. See text for further description of characters. References for each character state are indicated as superscript and are numbered as follows: 1= Abe and Munehara (2009), 2 = Bolin (1941), 3 = Bolin (1944), 4 = Hann (1930), 5 = Hubbs (1966), 6 = Jordan and Evermann (1898), 7 = Koya et al. (2011), 8 = Misitano (1980), 9 = Morris (1952), 11 = Petersen et al. (2005), 12 = Schultz (1938), 13 = Mecklenburg et al. (2002).

Group	Genus	Species	Character					
			1	2	3	4	5	6
Ingroup								
	Artedius	*corallinus*	0^{3}	0^{3}	?	?	?	?
	Artedius	*fenestralis*	0^{3}	0^{3}	?	4^{7}	1^{11}	0^{11}
	Artedius	*harringtoni*	1^{3}	0^{3}	1^{4}	1^{7}	1^{11}	1^{11}
	Artedius	*lateralis*	0^{3}	0^{3}	0^{4}	4^{7}	1^{11}	0^{11}
	Artedius	*notospilotus*	0^{3}	0^{3}	0^{4}	?	?	?
	Clinocottus	*acuticeps*	1^{3}	1^{3}	2^{4}	?	?	?
	Clinocottus	*analis*	1^{3}	1^{3}	2^{4}	?	$0^{1,5}$	$1^{1,5}$
	Clinocottus	*embryum*	1^{3}	1^{3}	2^{4}	?	?	?
	Clinocottus	*globiceps*	1^{3}	1^{3}	2^{4}	?	?	?
	Clinocottus	*recalvus*	1^{3}	1^{3}	2^{4}	3^{7}	$0^{1,9}$	$1^{1,9}$
	Leiocottus	*hirundo*	1^{3}	1^{3}	?	?	?	?
	Oligocottus	*maculosus*	1^{3}	0^{3}	1^{4}	1^{7}	0^{1}	1^{1}
	Oligocottus	*rimensis*	1^{3}	0^{3}	2^{4}	?	?	?
	Oligocottus	*rubellio*	1^{3}	0^{3}	2^{4}	?	?	?
	Oligocottus	*snyderi*	1^{3}	0^{3}	2^{4}	?	?	$1^{1,10}$
	Orthonopias	*triacis*	1^{3}	1^{3}	2^{4}	?	?	$1^{1,2}$
Outgroup								
	Chitonotus	*pugetensis*	1^{3}	1^{3}	?	?	?	$1^{1,8}$
	Icelinus	*filamentosus*	1^{3}	0^{3}	?	?	?	?
	Icelus	*spiniger*	1^{13}	1^{13}	?	?	?	?
	Phallocottus	*obtusus*	1^{12}	1^{12}	?	?	?	?
	Sigmistes	*caulias*	1^{6}	1^{6}	?	?	?	?
	Sigmistes	*smithi*	1^{12}	1^{12}	?	?	?	?

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Chapter 4: general conclusions

The first chapter of this thesis reviewed the systematics of species that have been historically allied with *Oligocottus maculosus*. These include all currently recognized species in the genera: *Artedius, Ruscarius, Clinocottus, Oligocottus, Orthonopias, Leiocottus, Sigmistes*, and *Phallocottus*. The first chapter also included a review of the history of generic affinities among the members of this group. In brief, *Clinocottus* and *Oligocottus* have been closely allied in virtually every study in which members of the two genera were included (e.g., Chapter 1: Figures 1.1-1.4, 1.7, 1.8), with the notable exception of Yabe (1985, see Chapter 1: Fig. 1.5) and Jackson (2003, see Chapter 1: Fig. 1.6). *Artedius* has been placed as sister to the *Clinocottus*-*Oligocottus* clade (Begle 1989, Ramon and Knope 2008, Taranets 1941, Washington 1986) or as part of an unresolved clade consisting of those three genera (e.g., Bolin 1947). *Ruscarius* has been historically lumped into *Artedius* (Bolin 1944), but recent analyses have placed it as a basal lineage to the *Artedius*-*Clinocottus*-*Oligocottus* clade (Begle 1989, Ramon and Knope 2008). *Orthonopias* has been repeatedly allied with *Artedius* (e.g., Bolin 1944, Bolin 1947, Taranets 1941). *Leiocottus* and *Sigmistes* have both been repeatedly allied with *Clinocottus* (Bolin 1944, Hubbs 1926, Taranets 1941). Finally, *Phallocottus* has been allied with *Sigmistes* and therefore, by extension, *Clinocottus* (Howe and Richardson 1978, Schultz 1938).

The second chapter was a phylogenetic study of the oligocottine sculpins using an extensive molecular dataset consisting of DNA sequences from eight different genomic regions. These loci included one mitochondrial protein-coding region, two nuclear introns, and five nuclear protein-coding regions. The loci were concatenated and partitioned by locus, with each partition free to evolve independently. Evolutionary relationships were inferred using parsimony, maximum likelihood, and Bayesian inference. The results of these analyses showed strong support for the inclusion of the genera: *Oligocottus*, *Clinocottus*, *Artedius*, *Leiocottus*, and *Orthonopias* within Oligocottinae. However, the results also showed that the genera *Sigmistes* and *Phallocottus*, which had historically been placed within Oligocottinae or allied to

Oligocottinae (respectively), are not closely related to other oligocottine sculpins, and should therefore not be included in the subfamily.

My third chapter was an analysis of the evolution of reproductive behaviors and associated morphological characters in members of Oligocottinae. These traits were taken from previous studies and mapped onto an ultrametric phylogeny inferred from the dataset generated in Chapter 2. Ancestral state reconstruction was used to explore the evolution the traits. The results showed that copulation and internal gamete association are likely the ancestral states of Oligocottinae. It is possible that as some of the oligocottine lineages transitioned from subtidal to intertidal habitats, they began to encounter a different suite of selective pressures than their subtidal relatives. It may be this rift that led the subtidal lineages to maintain parental care but transition away from copulation, while the intertidal lineages maintained copulation but quickly transitioned away from egg guarding to egg hiding.

The results of this thesis test, for the first time, the phylogenetic placement of the genera *Sigmistes* and *Phallocottus*, as well as the monophyly of Oligocottinae (*sensu* Hubbs 1926). This testing was done with the most robust molecular dataset of oligocottine sculpins to date in terms of the number of individuals sampled per species and the number of characters used to infer phylogeny.

The results of this thesis also offer new insight into evolution of reproductive modes in sculpins. Previous studies hypothesized that copulation had evolved independently in disparate groups (Abe and Munehara 2009, Munoz 2010). The results of this thesis clearly show that in oligocottine sculpins, copulation is the ancestral state, and has been secondarily lost in some taxa. This highlights the necessity of using a well-supported phylogeny when attempting to infer the evolutionary history of traits within a group. Given the diversity of reproductive strategies found among sculpins, the potential to explore complex patterns of evolution is great in this group. The forces that have driven the evolution of parental care and copulation in this group are certainly not unique. Understanding the ways in which these fishes have changed as a result of those forces

would undoubtedly shed more light on how these complex traits have evolved in other groups as well.

4.1 References

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